Cancer 2

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THE FOURTH REPORT

OF

# THE CAROLINE BREWER CROFT FUND CANCER COMMISSION

OF

THE HARVARD MEDICAL SCHOOL

BOSTON
MASSACHUSETTS
1907

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OF

## THE HARVARD MEDICAL SCHOOL

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FOURTH REPORT

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## INTRODUCTION.

J. Collins Warren.

In presenting the Fourth Report of The Caroline Brewer Croft Fund Cancer Commission, the committee wishes to call attention to the work which has been covered in previous reports, and to the different phases of the question of the causation of cancer which have been attacked.

The First Report was issued in 1900. At that time there were many questions of cancer etiology which demanded discussion and solution. The question of the parasitic origin of cancer obtained then a much more general credence than it does now. There were reports from various sources of microörganisms of one kind or another, which it was claimed played a prominent part in the cancer process. Prominent among these we may refer to the so-called "Plimmer's Bodies," the blastomycetic group of organisms, various bacteria, and the so-called sporozoa of certain animals. A proper consideration of these topics required a study of the field of work by a number of individuals, and the First Report accordingly contained papers upon several of these subjects by Drs. E. H. Nichols, R. B. Greenough, and E. E. Tyzzer. A series of experiments were also made by Dr. Oscar Richardson to determine whether any of the bodies found in the cells of carcinoma could be made to grow in nutrient media.

These investigations were continued in the Second Report, published in 1902. Studies were made of the coccidium infection in the rabbit, and of the histological appearances in the disease known as molluscum contagiosum. Investigations of the relation of blastomycetes to cancer were pursued still further, and the nature of the cell inclusions in cancer, which at that time caused much discussion amongst those engaged in this work, was studied and analyzed.

The Third Report appeared in 1905, and contained further contributions to the study of cell inclusions, and an extensive series of experiments upon the implantation of tissue in living animals. Professor F. B. Mallory made in this report a contribution to the classification of tumors by means of a study of cells and intercellular fibrils by special staining methods. The Third Report also entered somewhat into the study of the clinical side of the cancer question with an investigation of the extent to which the action of the Röntgen ray was of value from a therapeutic point of view.

The Commission feels that the work done thus far has been of considerable value, and that the conclusions deduced from the investigations made have withstood, in a most satisfactory way, the further test of time and discussion. It may be said that this work has aided in the settlement of many of the vexed questions which arose at the outset of the scientific investigation of this disease, and that by removing from further consideration some of the unsubstantiated theories of the parasitic origin of cancer, has contributed toward the ultimate elucidation of the great problem of the origin of this disease.

In the earlier reports the investigations of the Commission were confined almost entirely to the study of cancer from the pathological and histological point of view, and animal experimentation was employed only secondarily and with only negative results. The discovery by Professor Jensen of the inoculable carcinomata of mice, and the stimulus given thereby to experimental research upon cancer problems, opened a new field of activity which was promptly taken up by many commissions and institutions devoted to cancer research. It is to the results of this investigation that the Fourth Report is devoted.

By means of these inoculable mouse tumors the investigator is able to study the natural history of the disease, and to observe the cancer cell itself under various conditions as an actively growing parasitic organism. Not only can the relations of the cancer cells to the other tissues of the body and the different stages in the life history of the cell itself be studied, but the possible causes —

parasitic, chemical, or whatever they may be — which from time to time play a part in the phenomena of cell growth, may be approached. During the course of these experimental investigations, which have now become most extensive, many incidental data of extreme interest have been obtained bearing upon such topics as the natural or acquired resistance of the body tissues to infection with cancer cells, natural and acquired immunity, and even to the possible curative action of the blood or serum of recovered animals.

The Commission recognized the importance of approaching the subject of cancer research from an etiological point of view in the early stages of their investigations, but does not wish to be regarded as considering lightly the importance of the study of the disease from a clinical standpoint. To carry on clinical investigations on a scale which would bring out new and valuable data would require resources far beyond what most commissions have at their disposal. It is along these lines of investigation that large endowments would enable us to place the clinical and the laboratory work in close juxtaposition in such a way as to produce results which it is not possible to obtain either at the hospitals, or with the material of private practice which practitioners of the present day have at their disposal. Special wards for the study of cancer involve a large expenditure of money. The disease should be studied not only in its incurable stages, but at its very inception, when it is most susceptible to the action of remedial measures. Such institutions should be planned also to train men especially for cancer work. The Commission feels that the investigation of cancer has now reached a point where it is possible to take a new departure in this direction on a far more extended scale than has ever been attempted, and on lines even broader than those which have been laid down for the study and control of tuberculosis. Such institutions would not only be productive of valuable scientific data, but would train men who would be of the greatest value to the public health of the community from a protective standpoint. A moderate portion of the vast sums of money which are being used in the crusade against tuberculosis would be of the greatest value at the present moment in the attack upon perhaps the next most dreaded disease of modern times.

The report which the Commission presents in its fourth publication is based upon a series of observations and experiments conducted by Dr. E. E. Tyzzer, especially with reference to the inoculable tumors of mice. Concentration upon this subject has been made possible by the elimination of many other problems which at first presented themselves as has been detailed in previous reports, and the work has been greatly stimulated by the researches carried on in other laboratories and under other commissions along this line. The Commission is glad to acknowledge, gratefully, the assistance which it has received from other laboratories devoted to cancer research. Especially would it express thanks to Professor C.O. Jensen of Copenhagen; Dr. E. L. Bashford of the Imperial Cancer Research Commission of London; Professor Ehrlich of Frankfurt; Professor Borrel of the Institute Pasteur of Paris, and to Drs. Park, Gaylord, and Clowes of Buffalo.

# THE INOCULABLE TUMORS OF MICE.*

E. E. TYZZER.

The possibility of the artificial propagation of tumors, in certain animals, by the inoculation of tumor tissue into normal individuals of the same species was first shown in the experiments of Hanau,[15] who employed a carcinoma of a rat in his inoculations. In a similar manner, Morau [21] subsequently succeeded in propagating by inoculation an adeno-carcinoma of a mouse, and Loeb [17] a sarcoma of a rat. Jensen [16] in carrying on a series of inoculations with a carcinoma of a mouse demonstrated, by a histological study of the tissue taken at intervals after inoculation, that the epithelium of the tumor appearing in the inoculated animal is derived from the growth of the injected tumor epithelium, and not from the proliferation of the tissues of the inoculated animal. Bashford [1] made a thorough histological study of the changes taking place in and around the tumor tissue after its inoculation into normal mice, and confirmed Jensen's conclusions. Other investigators working upon this subject have become convinced that the development of these tumors in the animals inoculated is dependent upon the transplantation of tumor cells.

Adeno-carcinomata of mice have also been propagated by Borrel,[4] Haaland,[14] Ehrlich,[7, 8, 9, 10] Michaelis,[20] Bashford,[1, 2, 3] Loeb,[18] and others. These adeno-carcinomata are subcutaneous in origin, and have usually occurred in female mice. Ehrlich and Apolant [7] have obtained inoculable sarcomata which originated in their series of carcinoma inoculations. Although the original tumor was carcinoma, certain of the tumors resulting from the inoculations were of mixed type and consisted of both epithelial and connective tissue elements. These mixed tumors on further inoculation gave rise, in certain instances, to pure sarcomata. Loeb [18] had obtained similar

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* Received for publication Aug. 29, 1907.

results with a tumor of a Japanese waltzing mouse. Ehrlich[9] has also propagated a chondroma of a mouse. There is as yet no unity of opinion concerning the nature of the so-called infectious lympho-sarcoma which occurs frequently in dogs.* Some of the investigators are convinced that the tumor nodules arise from the proliferation of the tissue of the inoculated animal; others believe that the nodules arise from the growth of tumor cells which are introduced into the body of the normal animal by the act of inoculation.

The demonstration of the inoculability of certain tumors in animals has opened a new field of research in that an experimental basis is furnished for the investigation of various problems relating to tumor growth. Much has been done with the end in view of producing in animals an artificial immunity to the inoculable tumors, and results of considerable theoretical interest have been obtained. It has been found that in certain mice the inoculated tumors grow for a time, but later disappear spontaneously. Such mice are immune to subsequent inoculations. Various methods have been employed in attempts to reduce the vitality of the tumor tissue before inoculation so as to produce an artificial immunity. Michaelis has treated the tumor with chloroform before injection, and Clowes[6] has employed potassium cyanide, mercuric chloride, and other chemicals. Ehrlich produces immunity to the inoculable tumors by inoculating mice with a carcinoma of the hemorrhagic type, which grows in not over .2 per cent of the mice inoculated. The animals treated in this way are in nearly all cases protected against the more virulent strains of tumors subsequently inoculated. Ehrlich explains these results on the hypothesis of his "side chain" theory, and believes that, in these instances, an antibody is produced. The immunity brought about by the injection of one tumor apparently protects against all others. Thus he found that carcinoma inoculations protect mice against subsequently inoculated sarcoma and vice versa. Likewise tumor material injected into a mouse which already possesses a tumor invariably fails to grow. By inoculating rats with mouse carcinoma, Ehrlich found that the tumor epithelium grew for a time, but later disappeared. Rats injected in this manner developed an active immunity, and tumor epithelium subsequently inoculated failed to grow. If the tumor tissue, during its period of active growth in an inoculated rat, was transplanted into another rat, it failed to develop further, but if transferred back to a mouse it developed into a tumor. From these facts he infers that the rat tissues lacked an "X" substance which stimulates the tumor cells to active growth. This substance is found in the tissues of the mouse, and enough of this substance is carried over with the inoculated tumor tissue to enable it to grow for a short time in the body of the rat. This is demonstrated by the failure of the tumor tissue to grow when transferred to a second rat. Thus the rat, before it develops an active immunity, possesses what Ehrlich terms an "atreptic" immunity (*τρεφω*, to nourish).

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* Reviews of the literature bearing on this subject are to be found in the recent articles of Ewing and Beebe,[11] Bashford,[1, 3] and Borrel.[5]

It will be of interest to determine whether an artificially produced immunity to inoculable tumors serves as protection against the subsequent development of primary tumors. In a series of spontaneous tumors described in another section of this report, seven of the sixteen mice had been previously inoculated with tumor tissue of some sort. Whether the primary tumors in these cases were present previous to or developed after the inoculation — a point which, on account of their location, could not be determined — it appears either that the injection of mice with tumor tissue does not prevent the development of primary growths, or that the presence of primary growths in mice do not prevent successful inoculation. In two of the mice in which primary tumors were found, the inoculated tumors grew to large size.

Recent observations of Flexner and Jobling[12] indicate that certain properties of the inoculable tumors of mice may not be common to all tumors. They have worked with a sarcoma, inoculable in rats. This tumor grows when inoculated into rats which already possess tumors from previous inoculation. They also find that the growth of this tumor is increased by the injection of tumor juice into the animal.

Regarding the inoculation of tumor material into individuals of another species, most investigators have obtained negative results. Nichols[22] in the third report of the Caroline Brewer Croft Cancer Commission gives a comprehensive review of this question. All attempts made by him to transplant human cancers to rabbits and guinea-pigs resulted negatively. Mayet,[19] however, claims to produce tumors in dogs by the inoculation of human tumors. Since results such as these are infrequent, they require further confirmation by other investigators. Tumors are not infrequently met with in uninoculated animals kept for long periods of time under observation. There also appears to be some discrepancy as to what is to be regarded a tumor. Sanfelice[23] claims to produce tumors in animals by the inoculation of blastomycetes and their soluble products. The plates accompanying Sanfelice's article present no evidence that he is dealing with a true tumor, and it seems probable that the nodules which he obtains are infectious granulomata.

Opportunity is afforded, with animals kept constantly under observation, for obtaining data concerning the infectiousness of tumors. Tumors have occurred with such frequency in certain lots of mice often derived from a single cage that, to many investigators, it has appeared as evidence of infection. Such occurrences have been reported by Michaelis,[20] Haaland,[14] and Gaylord and Clowes.[13] Bashford,[1, 3] however, finds no evidence of infection in the development of tumors. He furthermore calls attention to the fact that the failure of tumors to develop in mice placed in cages in which tumor mice have been kept is not due to the absence of a suitable soil on which the virus may grow, for the exposed mice may by inoculation be demonstrated susceptible to the inoculable tumors. The increase in the virulence of certain inoculable tumors after being incubated for a time at temperatures from 38.5° to 41° C. as claimed by

Clowes[6] would appear to favor the presence of a living organism. No one, however, has been able to separate the hypothetical virus from the tumor cells, and, if a virus is present, it is destroyed by agencies which destroy the tumor cells. The evidence thus far furnished in favor of cage infection appears too meager to be conclusive. There has been no attempt on the part of the supporters of this theory to make thorough control observations, and the factor of heredity has been practically ignored. If mice are kept under observation for sufficiently long periods of time, tumors frequently appear among them even under conditions unfavorable to infection.

The following data, which I have obtained while keeping the Jensen and the Ehrlich "Stamm 11" tumors, are given in brief. No attempt has thus far been made to carry out inoculation experiments on such an extensive scale as has been done in other laboratories. The results, although meager are in the main confirmatory of those of other investigators. The results of the experimental inoculation of certain primary tumors, which have arisen spontaneously in mice kept under observation, are given in another section.

During the summer of 1905 mice inoculated with the Jensen strain of tumor were received from the Imperial Cancer Commission of Great Britain and from the Gratwick Laboratory for the Investigation of Cancer, Buffalo; a mouse inoculated with a Borrel strain of tumor from the Pasteur Institute; and mice with tumors designated "Stamm 11" from Professor Ehrlich. Both the Jensen and the Ehrlich tumors have been successfully transplanted from mouse to mouse and are growing at the present time. The mouse with the Borrel tumor died soon after its arrival, and the inoculation of the nodule which it presented into other mice was without result.

The technic at first employed in the inoculation of these tumors was to crush the tumor material in a small mortar, add a small amount of .6 per cent solution of sodium chloride so as to make it fluid, and then inject subcutaneously by means of a hypodermic syringe. This method was afterwards given up, and instead the tumor material was, without any addition of fluid, drawn into a small trochar and deposited beneath the skin of a mouse to be inoculated. In instances in which the tumor to be inoculated was infected with bacteria, it was found that by freezing it several times with carbon dioxide or by keeping it frozen over night, the tumor was still capable of growth, while the bacteria were apparently rendered inert. That the tumor epithelium is relatively more resistant than other tissues to low temperatures is readily shown by a comparative histological study of the other organs of the mouse frozen together with the tumor. All the organs show marked changes, and the liver tissue is scarcely recognizable. The frozen tumor epithelium, on the other hand, shows but slight change and appears to be living.

## I. Inoculations with the Jensen tumor.

The Jensen tumor has been carried, during this period of investigation, for ten generations or transplants and has invariably retained its original type. With the exception of a few cases, a histological study was made of all tumors produced by inoculation. Seventy-three tumors have resulted from the inoculations and of these eleven (sixteen per cent), after growing for a period, have diminished in size and have ultimately disappeared. Certain of these were taken for histological study when about to disappear.

The Jensen tumor is regarded by most observers as a carcinoma. Its epithelium is arranged in solid masses or lobules which present nothing suggestive of glandular structure. The epithelium in the interior of the lobules — that is, in those portions farthest from the source of nutrition — tends to undergo necrosis (Fig. 3). Tumors which have attained considerable size are usually largely necrotic. In these the necrotic portion most often occurs in the interior of the tumor nodule, where it appears as dry, cheesy material, while the living tumor tissue is found at the surface. In a few tumors, however, the living and necrotic tissues are intimately intermingled. The stroma of the tumor consists of a rather loose type of connective tissue. The tissue surrounding tumor nodules is often edematous and more marked inflammatory processes are not uncommon. These changes do not appear to represent a reaction to the growth of the tumor, but rather secondary processes attendant upon necrosis or bacterial infection of the tumor nodule.

Evidence of growth in tumor material may appear soon, or a long period after the inoculation. The mass deposited from the trochar is usually perceptible on palpation during the week following the inoculation. In cases where there is no inflammation about the inoculated mass it may from the first show a gradual increase in size. On the other hand, the injected mass may disappear within a fortnight or persist for a longer time and then disappear. In many such mice growth never takes place, but in others growth appears after a long period in which there is no perceptible mass or thickening at the site of the inoculation. The longest period observed between the inoculation and the first evidence of growth was sixty-four days. In this case a mass, developed on the back at the site of the inoculation, grew for a short time, and then disappeared. In several other cases the tumors first appeared five or six weeks after the inoculation. Of those tumors which appeared late, nearly all grew for a short time, then diminished in size and disappeared. There were two tumors, however, of which one appeared fifty-one days after inoculation, grew rapidly to large size, and produced metastases in the lungs; the other appeared sixty days after inoculation, and also attained great size. There were, also, several others which appeared four or five weeks after inoculation, and grew slowly, but eventually attained considerable size.

The rate of growth varies greatly. Tumors produced in a single litter of mice, all inoculated with the same tumor, show great variation, some of them growing rapidly while others may grow very slowly. Usually those tumors which appear early after the inoculation show the greatest rapidity of growth.

The factor of age has been considered in the growth of inoculable tumors. Tumors have been produced in both old and young mice by inoculation. Of three mice inoculated when twenty-one days old, one developed a tumor which first appeared sixty-four days later, that is, at the age of eighty-five days. The rapid development and quick maturity of mice make it a difficult matter to determine the susceptibility in early life, for the reason that these animals become mature before the tumor is given sufficient time in which to grow perceptibly. In the case above mentioned, it seems possible that some condition essential to the active growth of the inoculable tumor is lacking in the early life of the mouse. It is my experience that the inoculation of the tumor into vigorous young adult mice results in growth in a larger number of cases than in old mice.

Metastases were found in four of the seventy-three cases of Jensen tumors of this series. The occurrence of these metastases was as follows: A mouse (designated 321 C) which had been inoculated with the Jensen tumor was received from the Buffalo Laboratory. This mouse presented several subcutaneous nodules and microscopic metastases in the lung. Ten mice were inoculated with the tumor of this mouse (321 C) with the result that tumors developed in eight, of which three had metastases in the lung. The tumor in one of the cases with the metastases was inoculated into two susceptible mice and produced rapidly-growing tumors in both cases, with metastases in the lungs of one. Thus these four cases with metastases were derived directly from a single case with metastases. In each case in which metastasis occurred, the growth of the primary tumor at the site of inoculation was extremely rapid, attaining the size of a pigeon's egg within four weeks. The metastases were found when the four mice were killed, respectively, twenty-eight, forty-one, forty-six, and forty-nine days after the inoculation. The size attained by the metastases corresponds quite closely to the interval which had elapsed since the inoculation (Figs. 2, 4, and 5). Thus the metastases found in the case taken twenty-eight days after inoculation were very minute and consisted of groups of tumor cells occluding small blood vessels; in the cases taken forty-one and forty-six days after inoculation the metastases were apparent to the naked eye, although very minute; and in the case taken forty-nine days after inoculation the lungs were studded with nodules, the largest of which measured five millimeters in diameter.

In this series of Jensen-tumor transplantations, metastasis invariably resulted from the dissemination of the tumor cells by way of the vascular system. The tumor material was in each instance inoculated subcutaneously, and in most cases the lymph nodes adjacent to the tumor were examined histologically. In no case were they found to be invaded by the tumor. In the lung the constant relation of the metastatic nodule to the pulmonary arteries leaves no doubt as to the mode of conveyance. In certain instances emboli consisting of tumor epithelium were found within the blood vessels. The tumor tissue may grow for a time within the vessel forming a mold of its cavity. If the tumor material only partially fills the vessel, its surface becomes covered with endothelium. The tumor continues to increase in size, dilates the vessel to its utmost, and finally grows out through the wall of the vessel. In some cases the elastic laminæ of the wall of the vessel are separated by layers of tumor epithelium. Even in nodules of considerable size the vessels from which they grow are readily demonstrated. If sections are stained with an elastic tissue stain the walls of the vessel from which the tumor has grown will be found to be outlined by the elastic tissue (Fig. 6). After breaking through the wall of the blood vessel the tumor epithelium fills the perivascular lymphatic, but is not confined to any extent in this space, for, apparently on account of the slight resistance of the lung tissue, it grows by expansion in all directions. After the metastatic nodules attain a certain size they acquire a stroma and blood supply of their own. The central portion of the nodules commonly becomes necrotic, and the metastases are identical in character with the subcutaneous growth developing at the site of inoculation.

Certain breeds of mice were found insusceptible to the Jensen tumor. The occurrence of mice insusceptible to the inoculable tumors has been noted by various investigators. Some, however, have found that this natural immunity may be offset, after a time, by the adaptation of the tumor to the special breed of mice. Thus, where but few tumors resulted from the inoculation of a large number of these mice, subsequent inoculation of the tumors which were obtained into other mice of the same breed was attended with a greater number of successful results. The "virulence" of the tumor might thus be said to be increased.

As regards the immunizing effect of unsuccessful inoculations but little has been determined in this investigation. Thirty-nine mice in which the Jensen tumor material was absorbed after its first inoculation, on reinoculation with the same strain of tumor, furnish negative results in thirty-one, and growths which attained large size in eight. This percentage (twenty per cent) of growths following the reinoculation of mice in which the tumor material had been absorbed after the first inoculation is considerably lower than the percentage (45.6 per cent) of growths following the inoculation of unused mice. In all cases the amount of tumor tissue injected was small, and in a number of instances a long interval of time separated the two inoculations. It does not appear that the absorption of tumor tissue in small amounts, such as were injected, is sufficient to produce any marked degree of immunity.

Three mice first inoculated subcutaneously with an adenocystoma of the lung, which was absorbed without any growth, were later inoculated with Jensen's tumor, which grew in all three.

Five mice that had previously been inoculated subcutaneously with mouse kidney tissue were later inoculated with the Jensen tumor; large growths resulted in two of the five cases.

Of five mice inoculated with the Jensen tumor, after having absorbed subcutaneously inoculated placental tissue, one developed a large growth.

In order to obtain reliable results in the production of artificial immunity to the inoculable tumors, it is necessary to work with mice which are known to be susceptible, otherwise it cannot be determined whether the immunity is natural or acquired. It is also necessary to use, for the immunizing injections, tumor material which is incapable of growth. With this in view, Ehrlich has used a tumor which grows in a small percentage of mice, and both Michaelis and Clowes have treated the tumor tissue with chemicals when injecting for immunizing purposes. In the above manner Ehrlich is able not only to protect mice against subsequent inoculations with the same tumor, but also against inoculations of tumors of another type. Since definite results have already been reached in this line of investigation, I have, thus far, undertaken no extensive experimentation relative to immunization against the inoculable tumors.

## II. Inoculations with Ehrlich's tumor "Stamm 11."

This tumor has been carried for ten transplants. The percentage of successful inoculations (52.62 per cent) was slightly greater than was the case with the Jensen tumor. Notwithstanding that so much depends upon the susceptibility of the mice employed and the conditions of the inoculation, this tumor furnished more uniform results than the Jensen tumor. There is usually a gradual increase in the mass of the inoculated tumor tissue, and the tumors rarely make their appearance later than two weeks after inoculation. It is also very unusual for a tumor of this strain to disappear spontaneously after a period of unquestionable growth. Except in mice partially immunized by previous injections, this has occurred in but one instance.

The Ehrlich "Stamm 11" tumor usually shows more variation in structure than the Jensen tumor. In certain portions of tumors of this strain the epithelium is often arranged in solid masses, while in others it is gland-like in its arrangement. The structure of the growth, even in the more gland-like portion, is very irregular, and the spaces which correspond to the lumina of the glands often appear as irregular clefts showing that the epithelium is somewhat papillary in arrangement. As the tumors increase in size after inoculation into normal animals, the central portion usually becomes necrotic, and in large tumors there is often only a relatively thin layer of living tumor epithelium situated at the surface. The necrosis appears to be brought about by the failure of the supply of nourishment. The tumors of this strain are occasionally cystic, the cavities being filled with thin, slightly brownish fluid.

The rate of growth is fairly uniform, and there is much less individual variation.

The reinoculation of five mice, which had absorbed the tumor material previously injected, furnished actively-growing tumors in two, a tumor which grew for a time but later became absorbed in one, and no tumors in the remaining two.

An attempt was made to produce an immunity to the Ehrlich tumor by injections of the necrotic portion of tumors of the same strain. The dry, central portion of a large tumor was frozen and ground repeatedly until reduced to a homogeneous paste. Two large drops of this were injected subcutaneously into each of four mice. Six days later each mouse received a second dose of the same material. They were all inoculated with living tumor tissue fourteen days after the first protective injection. Of the four mice, two developed large tumors, indicating that immunity had not followed the injection of the necrotic tumor material.

Double inoculations with the Ehrlich tumor were tried in two instances. In one case the second inoculation was made fourteen days after the first. Tumors developed from each inoculation. In the other case the second inoculation was made forty-seven days after the first, from which a large tumor had grown. A nodule appeared at the site of the second inoculation, being about the size of a pea, three weeks later, and then diminished in size during a period of four weeks, and ultimately disappeared. The tumor resulting from the first inoculation, in the meantime, grew constantly.

Ten mice which had absorbed Jensen tumor material were reinoculated with the "Stamm 11" tumor. Six of these developed tumors, two of which ceased to grow, and were later entirely absorbed. In the other four mice the inoculation resulted negatively.

These experiments, as well as similar inoculations with the Jensen tumor, demonstrate that the absorption of small amounts of tumor tissue is often insufficient to protect an animal against subsequent inoculations of tumor tissue.

In the three mice received from Professor Ehrlich, the "Stamm 11" tumor was growing subcutaneously, and in one of these there were metastases in one of the lungs and in the liver. The presence of a nodule of the tumor in the latter organ is perhaps best accounted for by the growth of tumor epithelium from the lung nodule, which was of large size, into a pulmonary vein through which channel it could pass into the systemic circulation. In the liver the tumor is necrotic except a thin peripheral layer of living epithelium. The liver tissue where it comes in contact with the surface of the tumor is, for the most part, normal. At one point, however, there is a small group of necrotic liver cells. There is no flattening of cells or distortion of the normal structure of the liver suggestive of the exertion of pressure by the growth of the tumor, but the normal tissue seems gradually to have disappeared before the growth of the tumor epithelium. The amount of necrosis in the tumor is probably due to its failure to derive blood vessels of sufficient size for its nutrition from this organ.

Only one other case with metastases occurred in fifty-nine mice in which this strain of tumor grew on subcutaneous inoculation. In this instance the mouse was killed sixty-four days after inoculation. The metastatic nodules were situated in the lungs.

## SUMMARY.

In carrying on experimental inoculations with the Jensen tumor, it has been kept up for ten generations or transplants. Actively-growing tumors developed in seventy-three (45.6

per cent) of the normal mice inoculated, and in the others the inoculations resulted negatively. Eleven (sixteen per cent) of the tumors developed for a time, but later disappeared spontaneously. The reinoculation of mice in which the first inoculation had resulted negatively produced tumors in only twenty per cent. Some of the tumors of this strain appeared soon after inoculation, others appeared after a considerable interval of time. One tumor was not apparent until sixty-four days after inoculation. Many of the tumors, which appeared late, afterward diminished in size and disappeared, but one which appeared sixty days after inoculation attained great size. From the result of the inoculation in the latter instance, it is evident that it is possible for the growth of the tumor epithelium to be suspended for a long period of time and then to assume great activity. It would be important to determine whether such fluctuations are due to some inherent property of the tumor cells or to the character of the soil in which they grow.

The Ehrlich "Stamm 11" tumor has also been carried through ten transplants. Actively-growing tumors appeared in fifty-nine (or 52.6 per cent) of the normal mice inoculated. In only one was there spontaneous retrogression. Tumors of this strain show greater regularity of growth than the Jensen tumors. They appear soon after inoculation and their subsequent development is usually quite uniform.

The tumors of both the above strains are often largely necrotic. This is apparently due to the unrestricted growth of the epithelium irrespective of blood supply. The growth of the tumor is so great that it outstrips the growth of its blood vessels, and the cells which are farthest from the supply of nutrition become necrotic.

Certain breeds of mice were found to be more susceptible to the two strains of inoculable tumor than others, and the inoculation of mice from one source always resulted negatively.

Metastases were present in four of the seventy-three mice in which the Jensen tumor developed. These were all situated in the lung. Various stages in the growth of these metastases were presented, and the various sizes of the nodules correspond quite closely to the interval of time between the inoculation and the death of each animal. Metastases were also found in the lungs of one and in the lungs and liver of another of the mice inoculated with the Ehrlich tumor. The manner of dissemination of the tumor cells in all these cases is usually quite obvious from the constant relation of the metastatic nodules to the pulmonary arteries. The nodules always arise from tumor cells which have lodged in the branches of the pulmonary artery, and, if sections are stained in a way to bring out the elastic tissue, the relation of this vessel to the nodule can be clearly distinguished.

In no instance were the lymph nodes adjacent to the tumor involved, although in many they were in actual contact with the surface of the tumor. In this respect these inoculated tumors behave quite differently from the spontaneous tumors of the mouse, which not infrequently invade the lymph nodes.

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## DESCRIPTION OF PLATES.

### Plate V.

Fig. 1. — Tumor material situated beneath the skin of a mouse taken eight days after inoculation with the Jensen tumor. The greater portion of the injected tissue is necrotic, but the deeply stained layer at the surface of the mass consists of rapidly-growing tumor epithelium.

Fig. 2. — Metastases in the lung of a mouse killed forty-nine days after inoculation with the Jensen tumor. The tumor nodules, on account of their growth from the pulmonary arteries, are invariably grouped about the bronchi.

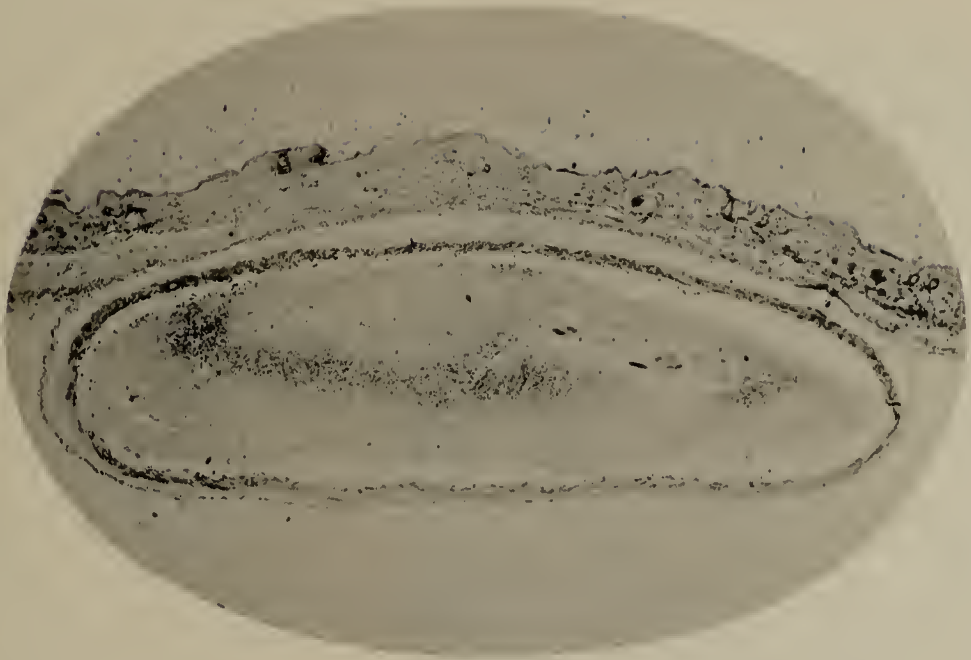
Fig. 3. — A section of a subcutaneous nodule of the Jensen tumor. The epithelium is arranged in alveoli, and portions farthest from the blood supply are necrotic.

## Plate VI.

Fig. 4. — Three small metastatic nodules in the lung of a mouse killed twenty-eight days after inoculation with the Jensen tumor.

Fig. 5. — Metastases of the Jensen tumor taken forty-one days after inoculation. The tumor epithelium has proliferated extensively within the pulmonary artery and is growing through its wall into the surrounding tissue.

Fig. 6. — From a section stained with Weigert's elastic tissue stain. The tumor tissue after occluding the artery has grown through its wall at one point. The wall of the vessel is outlined by elastic tissue.



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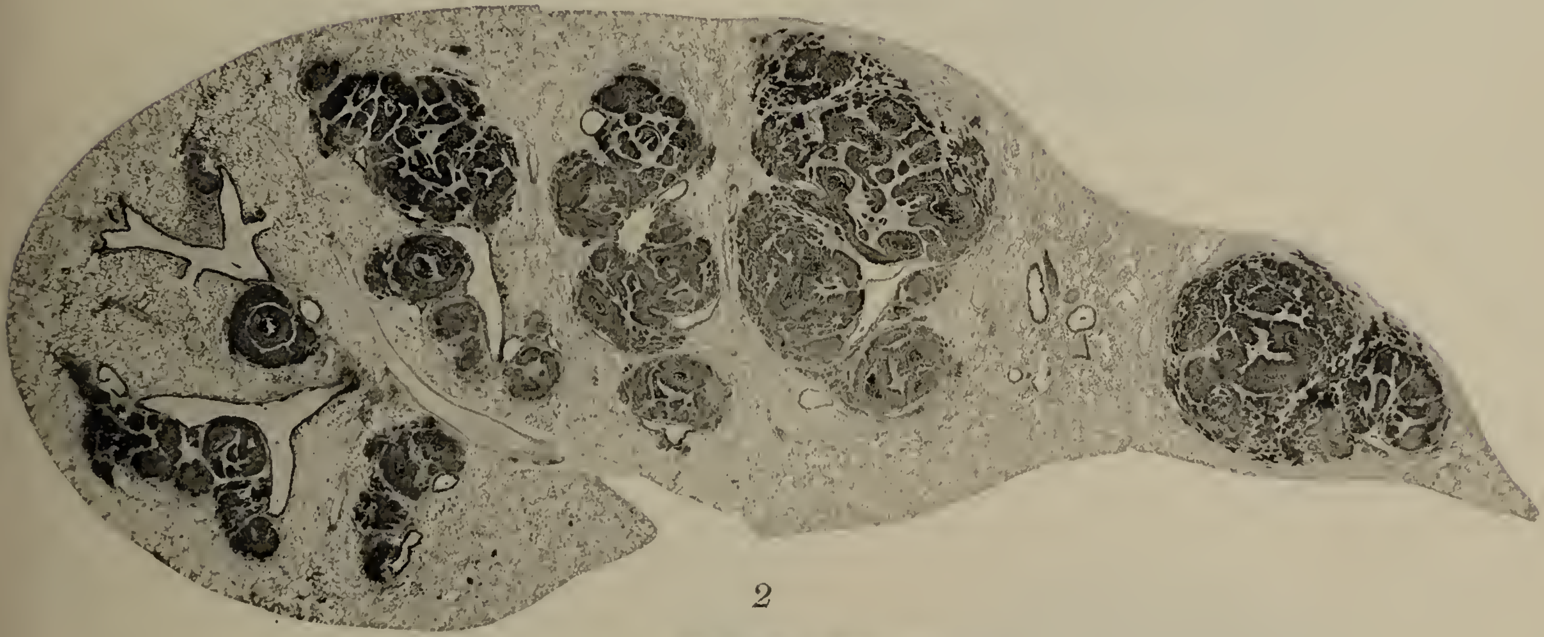

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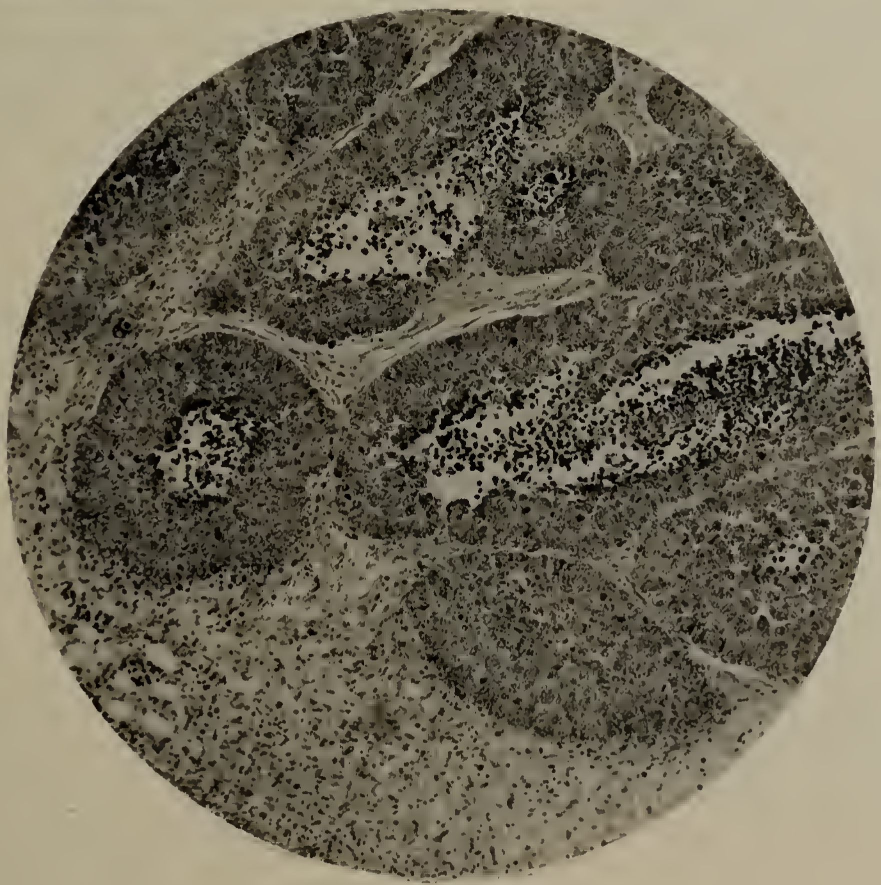

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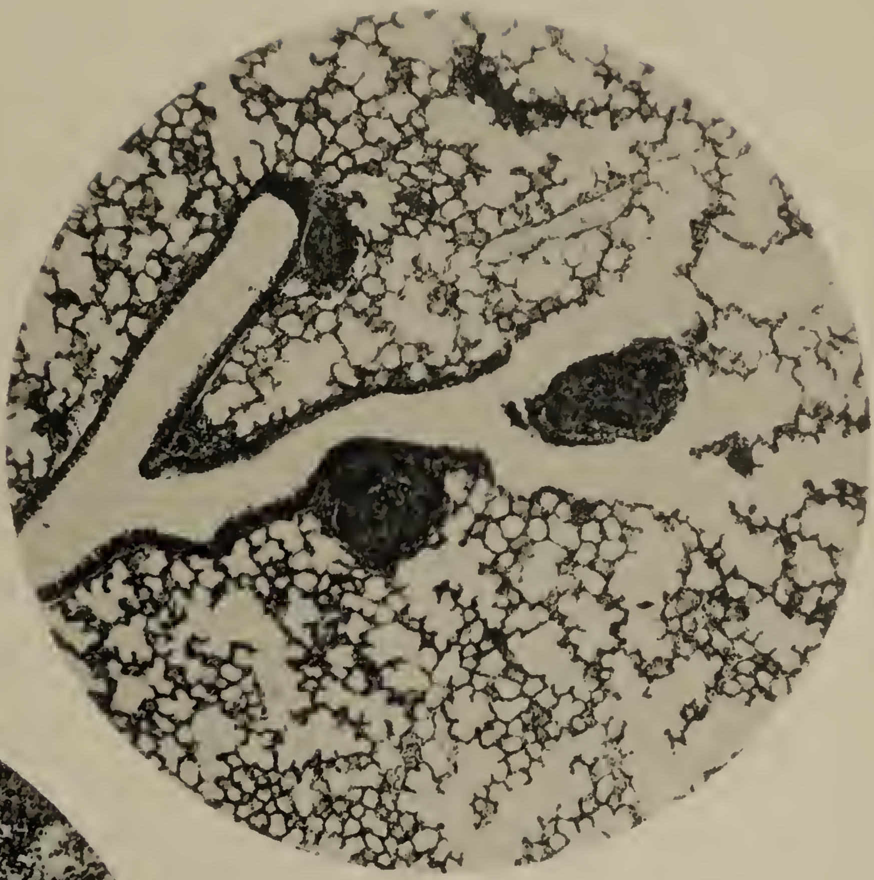

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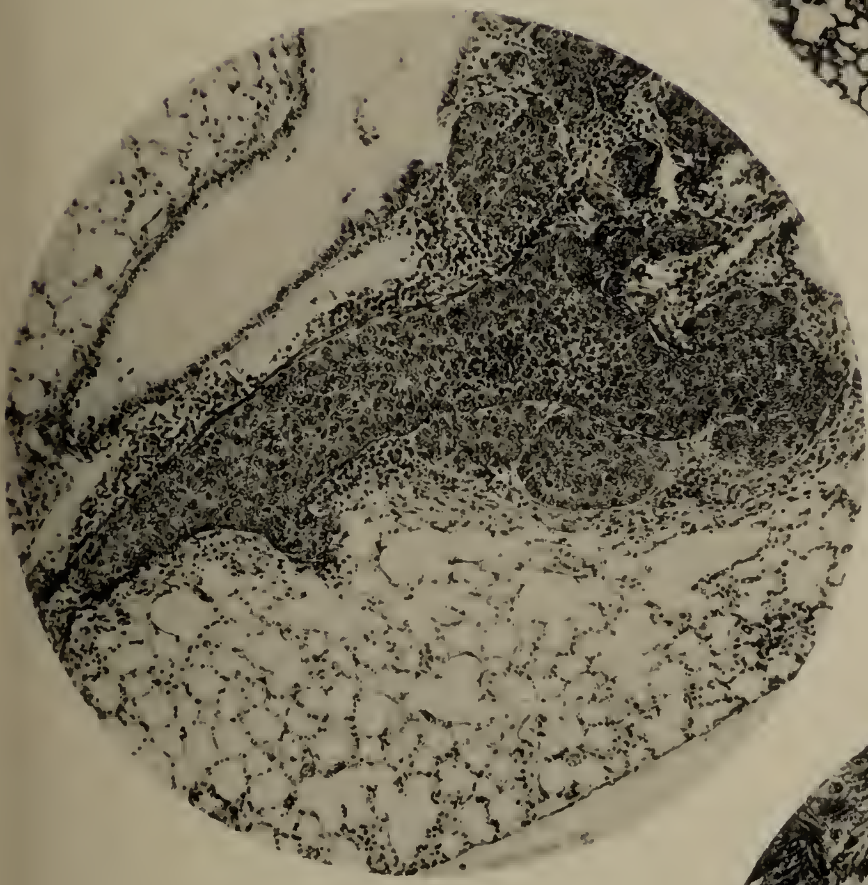

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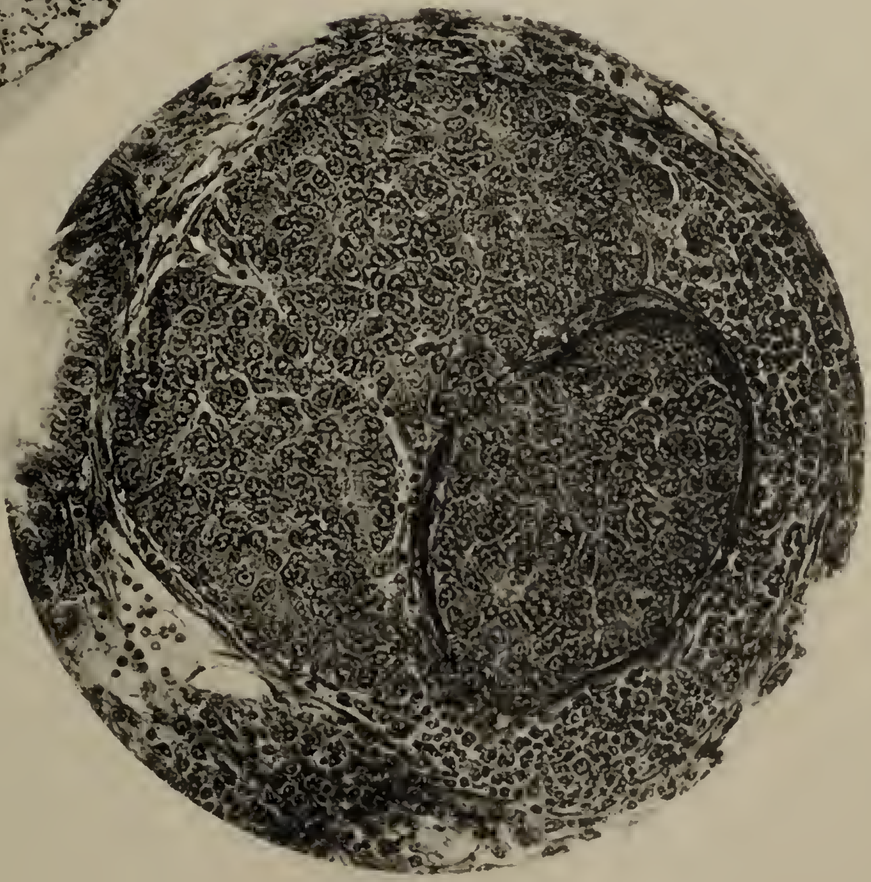

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# A SERIES OF TWENTY SPONTANEOUS TUMORS IN MICE, WITH THE ACCOMPANYING PATHOLOGICAL CHANGES AND THE RESULTS OF THE INOCULATION OF CERTAIN OF THESE TUMORS INTO NORMAL MICE.*

E. E. TYZZER.

In uninoculated mice, kept for breeding purposes, a number of tumors have appeared spontaneously. Also in a series of mice inoculated with tumor tissue, certain ones have presented primary tumors in portions of the body at a distance from the inoculation site. Since nearly all of the primary tumors which occurred in inoculated mice developed in the lung, it was impossible to determine whether they were present previous to inoculation or developed after it. Several mice, in which there were actively-growing inoculated tumors, presented also primary tumors of the lung. Other mice, in which the inoculated tumors either failed to develop, or developed for a short time and later disappeared, were found to have small tumors in the lung at the time of their death. One mouse inoculated subcutaneously on the back with the Jensen tumor presented subsequently no evidence of growth at the site of the inoculation, but a nodule appeared four weeks later in the mammary gland which proved to be a primary tumor. All these tumors are of distinctly different type from those used in the inoculations, and they undoubtedly represent true primary growths.

Since the various primary tumors have occurred with about equal frequency in both the inoculated and the noninoculated mice, there is no evidence that the inoculations stand in any causal relation to their development. Until such relation is shown, all primary tumors are regarded as spontaneous rather than experimental.

In the series of cases presented in this paper, the object is to give the most important points in the case-history, the pathological changes in the various organs, and the results

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* Received for publication Aug. 29, 1907.

of the inoculation of normal mice with the tumor tissue, as well as descriptions of the various primary tumors which have been met with.

### 1. Papillary cyst-adenoma of the lung.

Livingood[6] has described a tumor of the lung which occurred in a white mouse dying of an experimental bacterial infection. This tumor presented the appearance of an adeno-carcinoma, and seemed to originate from a bronchus. Within the bronchus it was found growing as a papilloma, at one point breaking through its wall, and growing into the surrounding tissue. He states that the greater portion of the tumor is made up of infiltrating fingers of epithelial cells.

Haaland[5] found nodules in the mouse's lung arising by the proliferation of the alveolar epithelium. Since these nodules were found in mice previously inoculated with tumor material, he suggests that they may have resulted from the presence of the inoculated tumor, but points out the necessity of distinguishing such nodules from the true metastases which originate with tumor cells that have lodged in the blood vessels.

Primary tumors of the lung have been found in twelve of the mice which have been under observation. In only one case did a tumor of this type attain sufficient size to interfere with the function of the lung. In the other cases the primary tumors appeared as minute nodules none of which exceeded four millimeters in diameter, so that their true nature was not apparent until sections were made.

Case No. 1.* — A black female mouse, apparently quite old, died three months after its purchase. This mouse was kept apart for breeding purposes, and was never inoculated. An autopsy was made immediately after death.

A grayish white mass measuring 5 x 10 x 10 millimeters is found situated in the superior lobe of the right lung. The surface of the mass, presenting to the lateral wall of the thorax, has a slightly depressed central portion and an elevated border. There are several smaller nodules in the same lobe and two very minute nodules in the middle lobe of the right lung. The kidneys present a puckered, uneven surface with many minute clear spots, evidently retention cysts, and scattered throughout the substance of the organ are many opaque whitish or yellowish points. Near the anterior end of the right kidney is a yellowish white nodule, one and a half millimeters in diameter, elevating the surface. The other organs present no gross lesions.

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* Full report of case in The Journal of the American Medical Association, 1906, XLVII., 1237.

A fresh teased preparation of the mass in the lung examined microscopically showed it to be an epithelial growth. Portions of this were placed in fixatives for histological study and the remainder inoculated into five mice.

The primary tumor of the lung has an alveolar structure and consists of epithelium which lines spaces made very irregular by projecting folds and processes of connective tissue. Different portions of the tumor vary considerably in structure. In certain portions the epithelial cells are much larger than in others. The central portion of the tumor has degenerated, and is largely replaced by connective tissue. There are no mitotic figures to be found, but direct nuclear fission is a prominent feature. Many of the nuclei present eosin-staining inclusions, which are often surrounded by a thin layer of deeply-staining material so that it resembles, somewhat, the nuclear membrane. The supporting tissue of the tumor is relatively small in amount and consists of both fibrous and elastic tissue, the latter element in relatively large amount. It is apparent that the tumor increases both by internal growth, — expansion — and by the growth of epithelium outward over the surfaces of the alveoli. The smaller tumor nodules are of similar structure. There is no evidence of metastasis by way of the blood vessels or lymphatics. It is possible that the dissemination of detached cells through the air spaces has been the mode of origin of the smaller nodules.

Certain portions of the lung about the tumor are atalectatic and it is here difficult to distinguish the exact limits of the latter. The alveolar walls are thickened and the lining epithelium is more or less cuboidal in type. Masses of cells filling the air spaces of the lung, both in portions adjacent to the tumor nodules already described and in portions at a distance from them, are difficult to interpret. These cells vary in size, but average much smaller than the epithelial cells of the tumor nodules. They also differ from the latter in that they stain less intensely and possess relatively small spherical nuclei. Whereas the epithelium in the definite tumor nodules is invariably found covering surfaces in a single layer, these cells, on the other hand, always tend to fill spaces.

They resemble very closely the swollen epithelial cells of the alveoli and it is by no means certain that they are not identical. The amount and arrangement of this epithelium is, however, very suggestive of a new growth (Fig. 5). In certain portions of the lung the normal structure is replaced by an irregular reticulum of connective and elastic tissue, in the interstices of which are masses of epithelial cells of this type. In the denser portions of this reticulum the epithelial cells are much flattened and distorted, while in the larger spaces they are polygonal. A few of these cells were found in mitotic division. In some instances they attain great size, and are then often multinucleated. Whether or not the masses of epithelial cells filling spaces in the lung represent a diffuse tumor growth, the condition certainly indicates a great proliferative activity throughout the lung.

The nodule situated in the kidney of this mouse consists of irregularly arranged epithelium with staining affinities closely resembling those of the convoluted tubules. The nodule is sharply defined and distinct from the kidney substance. It extends through the entire thickness of the cortex and bulges slightly from the surface of the kidney. The epithelium composing the tumor is columnar and in places is apparently ciliated. In portions of the nodule the epithelium has a tubular arrangement, while in other portions the growth is very irregular. Extending about a portion of the nodule there is a flattened cavity, making it appear probable that the growth has originated from the epithelium lining a cyst. Near the surface of the nodule is a mass of eosin-staining material consisting chiefly of dead epithelial cells. There are several long, pointed crystals, probably cholesterin crystals, in this material. The cortex of the kidney is much thinner than normal, and scattered throughout its substance are cysts of various sizes lined with swollen epithelium. Many of the tubules contain casts, and there are interstitial changes with collections of lymphoid cells and increase of connective tissue.

A portion of the lung tumor was snipped into small bits and inoculated subcutaneously into five mice. Following the inoculation a small mass appeared in each mouse at the site of inoculation. This was apparently due to exudation about the material inoculated, and a fortnight later it had entirely disappeared. In one mouse, sixteen days after the inoculation, there was a minute subcutaneous nodule which increased slowly in size and appeared as a rounded, slightly elevated translucent mass about three millimeters in diameter. Four weeks after its appearance this mass, which was evidently a small cyst, ruptured and practically disappeared, but after a few days it began to fill out again.

After rupturing a second time it finally disappeared about fourteen weeks after the inoculation. All five mice were under observation for the remainder of their lives, but nothing developed at the site of this inoculation. One and a half months after the inoculation with lung tumor three of the five mice, which were of a single litter, were inoculated with the Jensen tumor. In two of the mice this tumor grew rapidly and caused their death within two months after its inoculation. In the third mouse the growth of the Jensen tumor was very slow, and for long periods showed no apparent increase in size. This mouse, when killed, presented, in addition to the inoculated Jensen tumor, a small primary tumor of the lung. This is taken as Case No. 2 of this series.

The mouse, in which a small cyst had developed after inoculation with the lung tumor, died at the age of a year and five months and presented no new growth. The other mouse lived for eleven months after the inoculation with the lung tumor and developed no new growth.

Case No. 2. — A young gray male. This mouse, when about a mont old, was inoculated subcutaneously on the back with bits of the lung tumor of Case No. 1. As nothing appeared at the site of inoculation, the mouse was again inoculated about one and a half months later with the Jensen tumor. A month after this inoculation, a minute nodule appeared beneath the skin of the back and this was soon followed by a second nodule. These nodules grew very slowly, and at times were apparently stationary for weeks.

The mouse was killed accidentally when five and a half months old, and was autopsied sixteen hours after death. The inoculation of the two nodules of Jensen tumor into other mice was followed by growth in several cases.

In the posterior surface of the superior lobe of the left lung near its lower border there was a sharply circumscribed, grayish white nodule, about one and a half millimeters in diameter. It seemed probable that this nodule represented a metastasis of the inoculated tumor, but on study of sections it was found to be a tumor of another type. The other organs appeared normal.

In serial sections, the tumor nodule appears to be of the form of a broad based cone, the base of which is at the pleural surface and the apex of which is in close relation to a terminal bronchus. The tumor consists of epithelium situated upon irregular folds and processes of connective tissue. The epithelium consists of rather large cuboidal cells, arranged in a single layer, and possessing relatively small spherical nuclei. The cell-outlines are not prominent, but are distinguishable. No mitotic figures are found. The interstices of the tumor and the alveoli about it are filled with material, apparently a coagulum, in the form of delicately reticulated spheres such as are often seen with secreting cells.

Blood vessels extend into the tumor from various sides. The pleura covering the tumor is much thickened, and the elastic tissue is here present in a large amount. There is also a large amount of elastic tissue in the folds and processes upon which the epithelium is situated (Fig. 6). There is a tiny adhesion at the border of the lung, but no pathological process is found elsewhere.

In this case the tumor of the lung was of the same type as that used in the first inoculation, but, with the data at hand, it cannot be inferred that this tumor resulted from either of the two inoculations. Since it is of a type quite distinct from the Jensen tumor, which was growing subcutaneously in this animal, it cannot be regarded as a metastasis.

Furthermore, by its relations to the surrounding structure of the lung, it is shown to be a primary tumor.

Case No. 3 (1321). — A white male, which was used for breeding purposes, died at the age of one year and two months. The superior lobe of the right lung presents near its anterior border a translucent whitish nodule about one millimeter in diameter. This nodule consists of epithelium covering irregular folds of connective tissue, and is similar in many respects to those found in the two preceding cases. The central portion of the nodule is, however, almost solid, and here the epithelial cells are small and enmeshed in a reticulum of connective tissue. At the periphery of the nodule the epithelial cells are larger and are arranged somewhat irregularly so that they do not always form a single layer of cells. The supporting structure of the folds is so delicate that it requires special staining methods to bring it out clearly. A small bronchus terminates against this tumor, and the accompanying artery, which is not especially enlarged, ramifies through its structure. A few polynuclear leucocytes are present, scattered through the tumor, and in the surrounding lung tissue there are many large phagocytic cells filled with pigment. In another lobe of the lung is a minute focus of inflammation adjacent to which there is a parasite situated in the lymphatic vessel about one of the branches of the pulmonary artery. In section this parasite presents a body wall with short spines projecting from its outer surface. Within this is a group of bodies having the general appearance of ova. From both ends of each of these there project several small spines, and each contains nuclear material which is in some divided into few, in others into many separate masses.* The liver and spleen present no lesions of importance. The kidney is of normal size, and, considering the age of the animal, is in good condition. There are a few casts of blue-staining granular material within the tubules, and several dilated tubules. There is a small number of parasites, Klossiella muris, present and the remains of several worms encapsulated in dense fibrous tissue.

Case No. 4 (1301). — A white female mouse, one of the offspring of Mouse No. 3. This animal, when slightly over five months old, was inoculated with Ehrlich's tumor "Stamm 11." There was a slough at the site of the inoculation, but the tumor grew, nevertheless, and attained large size, killing the mouse about nine weeks after the inoculation. The central portion of the inoculated tumor was at this time necrotic. There was a minute whitish spot in the outer surface of the superior lobe of the left lung. In sections this is found to be a very minute tumor nodule, measuring .6 millimeter in diameter, and of the same type as was found in the preceding cases. Elastic tissue forms a considerable proportion of the supporting tissue of the tumor.

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* Borrel[2] has called attention to a worm which he observed in the lung of a mouse which possessed a tumor.

In the liver there are collections of cells, chiefly lymphoid and plasma cells, about the portal canals. The kidney contains large numbers of Klossiella muris and there are extensive interstitial foci. Many of the glomeruli are injected, and the epithelium of Bowman's capsule is thickened in many instances.

Case No. 5 (1385). — A white female mouse, offspring of Mouse No 3. This mouse was never inoculated. It died at the age of one year, three and a half weeks. A small tumor nodule was found in the middle lobe of right lung. The tissues were somewhat decomposed, but sections of the nodule show the tumor to be of the usual type.

Case No. 6 (1353). — A white male mouse, offspring of Mouse No. 3. Inoculated when two and a half months old with bits of a lympho-sarcoma of Mouse No. 11. Following this nothing appeared at the site of inoculation. This mouse always appeared pale and sickly. Died at the age of seven and a half months. A small tumor nodule was found at the inferior border of the superior lobe of the left lung. The folds and processes of this tumor, which was of the usual type, are somewhat compressed so that the nodule appears almost solid. A large dilated vein extends directly through this nodule. In the liver there is slight degeneration of parenchyma about the central veins. The kidney presents a proliferation in the epithelium of Bowman's capsules and several acute interstitial foci. There is also a slight infection with Klossiella muris. The spleen is small and presents no striking pathological process.

Case No. 7 (1544). — A black female mouse. This animal had never been inoculated. It died at the age of nine and a half months. The tumor in this case appeared as a minute translucent area, less than one millimeter across, situated in the outer surface of the superior lobe of the left lung. It corresponds to the usual type of lung tumors in mice, but in one portion the epithelium is composed of cells much larger than elsewhere and with an almost glandular arrangement. In a complete series of sections the tumor is not found to come in contact at any point with a bronchus. This is due to its flattened shape and its superficial situation at the surface of the lung.

Case No. 8 (1413). — Gray female. When four and a half months old this mouse was inoculated with the Jensen tumor. Two nodules appeared at the site of inoculation within four weeks after the inoculation, increased in size during the following two weeks, but afterward diminished in size and were absorbed in a short time. The mouse died at the age of ten and a half months, and presented a whitish, rounded nodule, two millimeters in diameter, near the lower border of the superior lobe of the right lung. This is found to be a tumor similar to those described in the preceding cases. Certain portions of the growth are, however, papillary in type. Elastic tissue is found in greatest amount in the older portions of the tumor. It occurs chiefly in the connective tissue septa, but is practically absent from the papillary portions of the tumor. In several places it has grown into bronchi where it retains its papillary type (Fig. 8). The interstices of the tumor, as well as the surrounding lung tissue, present material in the form of either fine granular precipitate or delicate rings.

Amyloid is found in the liver, spleen, and kidney. There is nephritis attended with extensive loss of cortical substance. In those areas where the process has been most severe, the tubules contain casts of deeply-staining material, and similar material is often found in a layer covering the glomerular tufts. Klossiella muris is present in large numbers.

Case No. 9 (1412). — A gray female mouse, born in the same litter with Mouse No. 8. Inoculated subcutaneously with the Jensen tumor when four and a half months old. One week after the inoculation there was a minute mass beneath the skin. This disappeared, and nothing further developed at the site of inoculation. The mouse died when eleven months old. Several grayish spots were noted in the lungs when fresh, but after fixation in Zenker's fluid a very minute nodule was found near the lower border of the superior lobe of the left lung. When sectioned this nodule is found to be a tumor of the type of the primary tumors of the lung previously found in other mice There is a large blood vessel extending through this tumor, but at no point is there any bronchus in contact with it.

Three other tumors of this type were found in addition to primary tumors of the mammary gland, in Cases Nos. 13, 15, and 16, which are given in full further on. The nodule in Mouse No. 13 is very minute and was discovered accidentally in stained sections of the lung. It is situated on a surface of a lobe which is in apposition to the surface of the lobe below. At one point the nodule unites with a bronchus. The tumor is composed of irregular folds covered with epithelium, but it does not form so definite a nodule as in the preceding cases. In Case No. 15 the nodule was apparent at the surface of the lung, but extends far into the lung where it has grown into a bronchus. The lung tumor of Case No. 16 is the only one in which mitotic figures have been found.

All the tumors of this series correspond to a single type although there are minor variations. They have thus far, in every instance, been situated at the surface of the lung, and are covered externally by pleura. They consist of epithelium situated upon irregular folds and processes of supporting tissue in which there is a relatively large amount of elastic tissue. There is invariably an increase in the elastic tissue of the pleura covering the surface of the tumor.

In some instances the plications of the tumor are separated by wide spaces so that there is considerable resemblance to the cystic new growths; in other instances the structure appears compressed and solid and the epithelial cells are situated in the meshes of a reticulum of connective tissue. Different portions of these tumors show great variation in structure and the epithelium may have either a gland-like or a papillary arrangement. It is a difficult matter to decide what name to apply to this type of tumor. It is not invariably glandular in structure, neither papillary nor cystic, yet for want of a better term it is designated "papillary cystadenoma."

The minute size of most of these growths makes it possible to determine quite accurately the site of their origin. They seem to bear no constant relation to the bronchi, although the latter often terminate at their surface. In Case No. 7 the tumor nodule is of such minute size and so situated that it is wholly independent of the bronchi. The relation of the tumor to the pleura seems to be quite constant, and, in all, the oldest portion of the tumor appears to be near the surface of the lung. The arteries from which these nodules derive their blood supply are branches of the pulmonary artery, and are often of considerable size.

The growth of most of these tumors is probably slow. Mitoses were found in only one instance. In many of the cases the tissue was not fixed until after the animal had been dead for some time, but in Cases Nos. 13, 15, and 16 the tissue was fixed immediately after killing, and in Mouse No. 1 soon after death. Mitotic figures were found only in the lung tumor of Case No. 16. On account of the rarity of indirect cell-division, it might be thought that these nodules were congenital and remained stationary throughout life. In one case, however, a growth of this sort was found filling a large portion of the right thorax, the existence of which throughout the life of the individual would be impossible. The growth of this tumor appears to have taken place in two ways — by internal increase through which the whole mass expanded, and by the growth of the epithelium over the surfaces of the surrounding alveoli, a process which is more or less analogous to the infiltrating growth of tumors outside the lung. In the same case there were also several smaller nodules of tumor in other portions of the lung, some of which are situated in other lobes. These smaller nodules did not occur in such relation to the blood vessels as to suggest a vascular dissemination of the tumor tissue. In Mouse No. 5 there were several small tumors situated in different lobes of the lung. It is possible that in these instances there were multiple primary tumors, or the smaller nodules may have originated with tumor cells which had been separated from the primary growth and distributed by way of the air passages. In two of the cases, the tumor had extended into the bronchi, so that its malignant character is unquestionable.

A tendency of the epithelial cells to degenerate is apparent in all the tumors of this type. Many cells contain two or more nuclei evidently resulting from direct fission. The nuclei may attain great size, are often irregular, and contain large hyaline inclusions. The retrograde changes are greatest in the central portion of the tumor and here the epithelium may be, to a large extent, replaced by connective tissue.

In Case No. 1 the lung presents, in addition to the definite tumor nodules, masses of cells filling the air spaces.

The cells which comprise them resemble very closely swollen cells of the alveolar epithelium. They differ from the epithelial cells of the definite tumor nodules in that they tend to fill spaces, whereas the latter always cover surfaces. Mitotic figures are occasionally found in these cells, but never in the epithelium of the more definite nodules in this case. Attention has been called in a previous paper * to the possible identity of these masses of cells and certain nodules which Haaland[5] has described as tumors arising from the alveolar epithelium in the lungs of mice previously inoculated subcutaneously with tumor material. I am now convinced that such is not the case, but that the nodules which he describes are, as he regards them, true adenomata.

It is possible that these masses of cells represent a diffuse form of tumor, but most of the evidence indicates a general proliferative activity on the part of the alveolar epithelium,

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* Jour. of the American Medical Association, 1906, XLVII., 1240.

possibly of the nature of a reaction to the primary tumor, which in this case attained a large size. It is not uncommon to find similar cells in chronic and acute processes in the lung. The cells in the present instance occasionally contain crystals, but show slight evidence of phagocytic activity.

Nine of the mice presenting lung tumors were females, and three were males. The age of these animals was not extremely great. The youngest was somewhat over five months, and the oldest, of known age, a year and two months. Six of the mice which presented tumors of this type had been previously inoculated, and six had never been inoculated with tumor tissue. It may be noted that the youngest mice which possessed these tumors were mice which had been inoculated. This may be explained by the fact that the chances for life are much greater in uninoculated than in the inoculated aninals.

Whether the injection of tumor tissue into the body of certain animals may in some way serve to excite certain tissues to irregular growth is a question upon which the present data throw no light. The possible influence of heredity in the development of these tumors will be discussed in another paper.

Minute tumors such as those presented in this series are easily overlooked, and it is probable that they are more common than is generally supposed. One tumor was not apparent at the time of the autopsy, but was discovered accidentally in a stained section. When small nodules are found in the lungs of mice which have tumors elsewhere, it is quite natural to conclude that they represent metastases. That such a conclusion may be erroneous is obvious from the occurrence, in certain of the above cases, of primary growths in the lungs of animals in which there was an inoculated tumor growing subcutaneously. If a histological study be made, there will be no difficulty in distinguishing a primary growth in the lung from a metastasis on account of the constant relation of the latter to the pulmonary artery or to the lymphatics.

### 2. Cyst-adenoma of the kidney.

Nodules were found in the kidneys of two mice. A description of one of these has already been given in Case No. 1, in which there was also a tumor of the lung.

Case No. 10 (1146). — A yellowish female, apparently quite old when purchased. This mouse was kept with the breeding mice, but had only one litter of three young. It always displayed a voracious appetite and devoured great quantities of food, but nevertheless remained rather thin. It lived for six months subsequent to its purchase, and was finally killed on being found moribund. There was a whitish nodule measuring two and a half millimeters in diameter in the posterior extremity of the left kidney a short distance from the hilus. The liver was small, and appeared to be less than one-fourth the normal volume for a mouse of this size. The lobes were rounded and somewhat constricted in places so that portions of the organ were pedunculated. The spleen was also of small size.

The nodule found in the kidney consists of a mass of irregularly arranged epithelium projecting into a cyst causing the latter to appear crescentic in section. The cyst is lined with epithelium, which is irregularly columnar in places, cuboidal or flattened in others, and forms a continuous, unbroken layer over the surface of the invaginating mass. The surface of the epithelium lining the cyst is everywhere uneven and groups of swollen, vacuolated cells form rounded, villus-like projections. The invaginating mass consists of epithelium similar to that lining the cyst, but is characteristically tubular in arrangement. It is also vacuolated as though it had contained fat droplets. The tubular arrangement is very irregular and in many places there are solid masses of epithelium. Several small cysts are found in the other parts of the kidney. There are small numbers of Klossiella muris present but not in the nodule.

In the liver the portal veins are dilated. There is degeneration and necrosis of scattered cells, usually most numerous in the central portions of the lobules. The capsule is much thickened at the points of constriction.

Groups of disintegrating cells are found in the spleen.

These nodules of the mouse's kidney appear to arise by the irregular proliferation of the epithelium lining cysts into the cavities of the cysts. Cysts in which the lining epithelium forms processes and folds which project into the cavity probably represent an early stage in the formation of these nodules (Fig. 10). The epithelium in the larger nodules has assumed an irregular tubular arrangement (Fig. 11).

In Case No. 10 the epithelial proliferation appears to have taken place outside the cyst and formed a mass which projects into the cavity of the cyst, but presses the cyst wall before it.

In neither of the two cases did the nodule present any evidence of active growth. No mitotic figures were found, but it is impossible to draw any conclusions as to the limitations of growth in these nodules. There is often considerable regenerative activity shown in the tubular epithelium of the kidney of the mouse. This is seen in cases of nephritis in which large portions of the kidney parenchyma are destroyed. It is possible that the nodules just described represent some such activity, although, in the second case, there was no destruction of kidney substance. Occurring together with a cyst-adenoma of the kidney there was, in Case No. 1, a primary tumor of the lung with other more general changes throughout the organ in addition to an advanced nephritis, and in Case No. 2 an atrophy of the liver which was apparently effected by a gradual destruction of liver cells.

### 3. Lympho-sarcoma.

Haaland[5] has described a malignant lymphoma in the mouse. In his six cases there was a great increase in the lymphoid tissue throughout the entire body. He, however, considered this increase to be of the nature of a general hypertrophy or hyperplasia of preëxisting tissues rather than of the nature of a neoplasm. Inoculations gave negative results, but, after placing normal mice in the cage with the mouse having a lymphoma, five similar tumors appeared in the space of two years.

The following two cases differ from Haaland's cases in that they present a well defined primary growth without appreciable hyperplasia of lymphoid tissue elsewhere in the body.

Case No. 11 (1350). — A yellowish brown female, which lived a year and a month after its purchase. It raised during this time five litters of young. When the last litter was well grown, a soft, spherical nodule, slightly over one centimeter in diameter, was found in the right inguinal region. This increased in size during the following period of ten days, at the end of which it was removed by operation. It consisted of a sharply circumscribed, homogeneous mass, the tissue of which appeared rather translucent with a pinkish tinge. Portions of the tumor were placed in various fixing fluids and the remainder used in the subcutaneous inoculation of seventeen mice. The mouse appeared weak after the operation, and, when seven days later breathing became labored, it was killed and autopsied at once. The operation wound appeared clean, but had not healed. The lungs present ill-defined grayish areas. The liver appears normal. The spleen is small and dark red. The posterior extremity of the left kidney is occupied by a large cyst, one centimeter in diameter, and filled with clear fluid. The left inguinal and axillary lymph nodes are small. In the mesentery is a mass of lymphoid tissue, five millimeters in length, appearing reddish and mottled so that it resembles somewhat splenic tissue. The skin is thin, and easily torn.

The rounded mass which was found growing in the inguinal region consists chiefly of small round cells. It contains very little connective tissue, but is permeated by a network of small blood vessels and capillaries, which show the large nuclei and spur-like processes characteristic of growing blood vessels. It is slightly less vascular in its center than near the surface. The cells, of which the mass is chiefly composed, are of the type found in the germinative centers of lymph follicles. The nuclei are considerably larger and more vesicular than the nuclei of lymphocytes of the type most commonly found in the blood. The cytoplasm is indefinite in character, and the cell outline is indistinct so that it can only be made out in certain areas of the section. Usually the cytoplasm of these cells appears as an indefinite, loosely reticulated material lying between the nuclei. Mitotic figures are numerous, and these, so far as can be ascertained, are of the normal type. The tumor mass is homogeneous throughout and there is no lobulation or tendency toward arrangement in follicles. Large phagocytic cells are numerous in certain areas near its surface. Many of the perivascular lymph spaces are filled with small lymphocytes, which are much smaller than any of the cells found elsewhere in the tumor. Portions of this tumor were impregnated with silver, following the technic of Levaditti,* in order to determine if spirochetæ were present. None of these organisms were found.

Most of the lymph nodes of this mouse, which was killed one week after the removal of the tumor, are small and present no prominent germinative areas. The lymph nodes are composed chiefly of cells of the plasma-cell type; that is, they possess a small rounded nucleus with the chromatin in dense masses and a relatively large amount of cytoplasm which stains bluish or lilac, and presents a definite cell contour. Mitotic figures are numerous. Many of these cells are vacuolated, others are filled with hyaline globules, and in places there are many disintegrated cells. The large mass of lymphoid tissue found in the mesentery appears to be a greatly enlarged lymph node. The sinuses contain blood, and the lymphatic cords present large numbers of polynuclear leucocytes. There are many giant cells such as those found in the bone-marrow and spleen.

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* Levaditti, C. L'histologie pathologique da la syphilis héréditaire. Anal. de l'Inst. Pasteur, 1906, XX., No. 1.

There is an active leucocytogenic bone-marrow.

Portions of the lung contain large numbers of irregularly rounded, lilac-staining bodies, possibly of the nature of corpora amylacea. There are also irregular, laminated deposits of material resembling fibrin in its arrangement situated in the lung tissue and long, slender crystals in the bronchi. These portions of the lung are filled with large phagocytic cells many of which contain greenish pigment or small crystals. There are many collections of lymphoid and plasma cells in the pleura and several fibrous adhesions.

The liver presents collections of cells of the plasma-cell type about the portal canals, but no other lesion.

The spleen contains large numbers of cells of the plasma-cell type and mitotic figures are numerous. The organ contains only a small amount of blood.

The large cyst present in the left kidney is lined with thin, flattened epithelium. There are many hyaline casts and cystic dilatations in the tubules in both kidneys. Many of the glomeruli are filled with hyaline material presumably amyloid. Klossiella muris is present in small numbers.

The mammary gland is filled with secretion and appears normal for this stage of its activity.

Nothing appeared at the sight of the inoculation in any of the seventeen mice inoculated subcutaneously with this tumor. The injected material could not be found in one mouse of this series which died seventeen days after the inoculation, so that it must have been absorbed at once. Nearly all of these mice lived for several months. One mouse of this series presented a minute tumor of the lung (see Case No. 6).

In this case there is in the inguinal region a definite primary tumor composed of tissue resembling lymphoid tissue. Since no inguinal lymph node was found in its vicinity it seems not improbable that it has replaced the latter structure. The tumor mass consists of small cells similar to those which compose the germinative areas of lymph follicles, a system of small blood vessels permeating its whole structure, and a relatively small amount of connective tissue most of which is situated about the blood vessels. Certain portions of the tumor contain numerous phagocytic cells. The tumor is limited by a delicate capsule of connective tissue, but none of the surrounding tissue was removed at operation, so that it was not determined whether this was invaded. Since the mouse died one week after the operation, nothing could be determined concerning recurrence.

The study of the various organs and tissues reveals no evidence of the dissemination of the tumor into other parts of the body. There is a chronic pleuritis and a chronic process in the lung associated with calcareous deposits. There is also a slight nephritis with what is probably amyloid deposited in the glomeruli. The lymphoid tissue throughout the body, although not increased, is somewhat modified, and cell differentiation appears to be greatest towards the type of the plasma cell. A lymph node of the mesentery is the only one which shows any appreciable increase in size. This is infiltrated with leucocytes and contains hemorrhage as well.

The inoculation of this lympho-sarcoma into seventeen normal mice resulted negatively, although several were the offspring of the mouse in which the tumor developed.

Case No. 12 (1715). — A black male about five and a half months old. Derived by mating a member of Family A (see section on Heredity), with a mouse of the Bashford strain. Died suddenly after a few convulsive movements.

There is a large mass situated in the thorax just anterior to the heart and apparently originating in the mediastinum. It is pale pink in color, and is slightly lobulated, although its surface is in general smooth. It measures 1.4 x .9 x .5 centimeters. There are numerous smaller nodules about the roots of the lungs and in the pericardium. Minute elevations are apparent on the surface of the lung. There is an escape of blood as the thorax is opened.

The tumor was not sectioned until after it had been fixed in Zenker's fluid. There then appeared to be softened areas in its interior.

Histologically the tumor is of the type of that described in the preceding case. It consists of small cells similar to those of the germinative centers of the lymph follicles, and possesses scarcely any stroma. The tumor is quite vascular and there are beautiful examples of growing blood vessels. In certain places there is hemorrhage into the tissue, in other places there are large numbers of disintegrating tumor cells and large phagocytes loaded with cell detritus. Mitotic figures are very numerous. The nodules observed in the pericardium consist of tissue similar in every respect to that found in the large tumor mass.

The tumor is found growing rather diffusely in the pleura, sometimes in thin strands, at other times forming irregular nodules. All the large arteries and veins of the lung are surrounded by tumor. The walls of the alveoli are somewhat thickened in certain portions of the lung. One portion of the lung presents a chronic inflammation with large numbers of phagocytic cells filled with eosin-staining needle-shaped crystals.

There is no hyperplasia of the lymphoid organs elsewhere in the body. None of the other organs presents any important lesion. The liver appears practically normal. In the spleen there is a marked degree of erythrocytogenesis. The stomach and the kidney contain small numbers of the usual parasites. The pancreas and brain appear normal.

The lympho-sarcoma originating in the mediastinum of this mouse possesses a structure practically identical with that of the tumor of the preceding case. The tumor in the present instance, however, had produced metastases in the pericardium, and had extended along the lymphatics accompanying the large vessels throughout the lung. Judging from the number of mitotic figures present, its growth was rapid. There are minute hemorrhages into its substance in places. In the interior of the mass there are many disintegrating tumor cells, and these are being taken up by the large phagocytes which are present.

### 4. Subcutaneous adeno-carcinoma.

Of the neoplasms which develop in mice, the epithelial tumors originating in the subcutaneous tissue have received most attention. By series of inoculations many have been artificially propagated for long periods of time. It is probable that many of these tumors originate in the mammary gland. They usually arise on the under surface of the body or on the sides, and occur most frequently in females, especially in old females which have been kept for breeding purposes. Various types of epithelial tumors occur.

Michaelis[8] has distinguished three types of epithelial tumors: 1. Cancer, the epithelium of which is arranged in alveoli, with little or much connective tissue according to the age of the growth. The Jensen tumor is an example of this type. 2. Adeno-carcinomata, which have a glandular structure, and present ducts and alveoli with regular cubical epithelium. Certain tumors of this group show a tendency to cyst formation through the dilatation of the glands. Others have a papillary structure through the uneven growth of the gland epithelium. 3. Tumors, the epithelium of which is divided by the connective tissue into large alveoli. In gross appearance these resemble tumors of the first type. Each alveolus is, however, subdivided into smaller groups.

Haaland[5] has described four types of epithelial tumors: 1. Adeno-carcinomata (tumors of the Parisian type). Metastasis takes place by way of the blood vessels. Neighboring lymph nodes are not invaded. 2. Tumors of the Jensen type. 3. Epidermoid carcinomata occurring on the jaw and invading the neighboring lymph nodes. 4. A molluscoid tumor, with lobules arranged as radiating rays with rounded bases at the periphery, from its structure possibly related to the hair follicles.

The most complete classification of epithelial tumors is given by Apolant.[1] The epithelial tumors of subcutaneous origin are divided into two groups, adenomata and carcinomata. With these two groups he distinguishes the following types:

1. Adenoma simplex. These are encapsulated, solid tumors having a lobular structure. The alveoli correspond very closely with those of the normal mammary gland, but are closely packed. In certain respects they resemble goitre. In the youngest tumors there is often no sharp demarcation between tumor and mammary gland. These structures appear to arise by hypertrophy of portions of the mammary gland.

2. Cyst-adenoma simplex. Such tumors are essentially similar to the preceding, the only difference being found in the dilatation of the lumina of the glands by secretion. This fluid may appear normal or more serous than normal.

3. Adenoma cysticum edematosum. In tumors of this type there is degeneration of the connective tissue between the alveoli, and the epithelium lining the cysts may also become degenerated, making the interpretation of the growth difficult. An additional factor is found, in some instances, in the condition of the blood vessels. The capillaries may

be so dilated that the structure resembles a cavernoma. Hemorrhage may occur producing adenoma cysticum hemorrhagicum.

4. Cyst-adenoma papillare. In instances in which the proliferation of the epithelium exceeds the cyst formation, papillæ are formed. The stroma may be small in amount or abundant.

1. Carcinoma simplex alveolare. In many adenomata the epithelium, in certain places, proliferates and grows wherever it finds space. Carcinoma may develop from the hemorrhagic cystadenomata. These tumors (cystocarcinoma hemorrhagicum) present great irregularity in structure and are usually poor in stroma. They form a large percentage of all mouse tumors, and are relatively benign.

2. Carcinoma papillare, including the "spalten bildendes" carcinoma. These tumors arise from the papillary cyst-adenomata.

Apolant states that whereas, in man the transition between adenoma and carcinoma is rare, in the mouse it is frequently observed.

Loeb[7] has described a tumor in a Japanese mouse, which presented two varieties of epithelium — cubical epithelium forming alveoli filled with colloid material, and epithelium possessing finely or coarsely vacuolated cytoplasm. The latter cells disintegrate to form spaces filled with fluid. The structure of this tumor suggests some relationship to the salivary or sebaceous glands.

The tumors presented in the following four cases are all of different types. They resemble quite closely mouse tumors described by other authors.

Case No. 13 (1415). — A black and white female, of breed obtained from Dr. E. F. Bashford. This mouse, when about ten months old, was inoculated subcutaneously on the back with Jensen tumor tissue. Nothing appeared subsequently at the site of the injection. Four weeks after the inoculation the left axillary lymph node was enlarged, and, one week later, a small nodule in the left inguinal region and a larger rounded oblong mass behind the right axilla were discovered. The enlarged left axillary gland later on appeared spherical and translucent, and had evidently become cystic. The mass behind the right axilla subsequently became harder, but showed increase in size. The mouse was killed about four weeks after the enlargement of the lymph nodes was first noted.

In the left axilla is a cyst filled with thin, clear, pale yellow fluid. In the mammary tissue, posterior to right fore-leg, is a flat nodular mass, measuring about one centimeter in length, and of whitish color, except one portion which is dull pink and resembles lymphoid tissue. Bits of this mass, examined microscopically, showed an epithelial tumor, and a portion was accordingly used to inoculate ten normal mice. Extending from this mass towards the axillary lymph node is a whitish cord of tissue evidently an obstructed lymphatic vessel. The mammary gland about the mass presents many opaque whitish granules. The lymph nodes about the lower jaw are greatly enlarged, probably due to inflammation about the lower incisors. The pancreas appears as a rounded nodule attached to the mesentery. The uterus contains several embryos measuring eight or nine millimeters in length and several smaller and probably abnormally developed ova. The histological findings are as follows:

The cyst, which was situated in the left axilla, is found to consist of a lymph node distended with clear fluid in which are several lymphoid cells and polynuclear leucocytes.*

Sections of the nodule from the right axilla show a large collection of lymphoid cells surrounding a mass of carcinoma tissue.

The lymphoid cells are intermingled with fat cells and mammary gland.

The included tumor consists of epithelium lining several large spaces, which are filled with eosin-staining, granular precipitate, and desquamated and necrotic tumor cells. The tumor epithelium is very irregular, but in many places shows a gland-like arrangement. Many of the degenerating cells are flattened and squamous in character, and, where they are compressed in small spaces, appear as epithelial pearls (Fig. 16).

In these the cells at the center stain deeply with eosin, while the surrounding cells contain keratohyaline granules, and resemble the cells of the stratum granulosum of the epidermis. Mitotic figures are not numerous in the tumor epithelium and certain portions are wholly necrotic and surrounded by giant cells. There is an area of marked edema in the connective tissue near the tumor, and several mammary gland ducts, in the same area, are dilated. The surrounding tissue is very vascular. Polynuclear leucocytes are nowhere present in large numbers, so that the reaction of the tissue appears to be much of the nature of the reaction about a foreign body.

The cord of tissue extending from the mass just described toward the axilla consists of foreign-body giant cells and lymphoid cells surrounding necrotic tumor epithelium. In places there are collections of polynuclear leucocytes, and the whole is very vascular.

The nodule situated in the left inguinal region is found to be a small primary tumor, of which the various portions differ greatly in character. It is situated against the inguinal lymph node which indents it slightly so that it is more or less cup-shaped.

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* Cystic nodules have occurred quite frequently among the mice under observation. They appear as swellings beneath the skin and have in several instances been mistaken for tumors.

The tumor consists, for the most part, of closely packed tubular glands. In some portions the glands are of uniform type, but in other portions the epithelium, instead of being arranged in a single layer, is greatly thickened and grows irregularly. In the central portion of the tumor, the epithelium is growing in large solid masses and presents all the characteristics of the carcinoma. Glands dilated with fluid form cysts in various parts of the tumor. Mitotic figures are numerous throughout the tumor, but are somewhat less numerous in parts showing the uniform glandular structure than in parts where the epithelium is growing in solid masses. A delicate network of connective tissue forms the stroma of the tumor. The adjacent inguinal lymph node is not involved.

A very minute tumor, which was overlooked at the time of the autopsy, is apparent in sections of the right lung. It measures about .8 millimeter and is situated in the lung substance and against the pleura in that portion of its surface which is in apposition with the surface of the adjoining lobe. It is of the same type as the other primary tumors of the lung, described in the preceding series, and it is distinct from the tumor of the breast which was found in this case, so that it cannot be considered a metastasis of the latter. The lymphatics around many of the branches of the pulmonary artery are filled with lymphoid cells.

The portal veins of the liver are greatly dilated. There are a few small collections of cells of the lymphoid and plasma-cell type about the portal canals and scattered elsewhere.

The spleen, which is large, shows erythrocytogenesis in a marked degree. Areas corresponding with the lymph-follicles are composed of tissue such as composes the germinative centers and this is also found distributed rather diffusely through the organ. Leucocytogenesis is nowhere in evidence. Disintegrating cells are found in moderate numbers. The pancreas appears normal.

The kidney contains a small number of Klossiella muris, but, except for a few minute acute interstitial foci, is otherwise normal.

The lymph nodes throughout the body show considerable activity and the germinative areas form a prominent feature. The submaxillary lymph nodes present the greatest degree of hyperplasia probably on account of an inflammation about the lower incisors.

The mammary gland appears normal and active.

Ten mice were inoculated subcutaneously on the back with bits of tissue from the mass situated posterior to the right fore-leg. In all of these there was ulceration and sloughing at the site of the inoculation. The tumor failed to develop in any of the inoculated mice. The mass from which the inoculations were made was later found to be a metastasis surrounded by a large amount of inflammatory tissue.

In this case a primary adeno-carcinoma (carcinoma simplex alveolare), situated in the inguinal region, with metastasis to the axillary region of the opposite side, and a papillary cyst-adenoma of the lung were found in a female mouse, aged ten months, a short time after it was inoculated with the Jensen tumor. The primary tumor situated in the inguinal region is undoubtedly of mammary origin. A portion of the tumor is composed of glandular tissue of the mammary gland type, but this is quite distinct from the normal gland. While portions of this tumor present the structure of a simple adenoma, other portions consist of epithelium growing irregularly after the manner of a carcinoma, so that transition from adenoma to carcinoma is seen in many places.

A mass of tumor tissue occurring in the axillary region, where it is invading the lymphatics, is considered to be a metastasis of the primary adeno-carcinoma of the inguinal region, although certain features which it presents are not shown in the latter. The inflammation about this metastatic nodule and also the degree of degeneration which its epithelium presents possibly account for its failure to grow when inoculated into other normal mice.

A minute primary tumor was found in the lung of this mouse. That this does not represent a metastasis from the mammary tumor is shown both by its distinct structural characteristics and by its relation to the tissue of the lung. It is similar to the other papillary cyst-adenoma of the lung already described.

The inoculation of this mouse with the Jensen tumor a short time prior to the finding of these tumors is another factor to be considered. There can be no question, however, as to the primary character of both the tumor of the lung and the adeno-carcinoma of the inguinal region. The tumor in the axillary region had grown into the lymphatics and showed also peculiarities, such as epithelial pearls, which have not as yet been observed in the Jensen tumor. Furthermore, it appeared at some distance from the site of the inoculation.

Except for the inflammation about the lower incisor teeth and a more or less general hyperplasia of the lymphatic tissue, there were no important changes in the other organs.

Case No. 14 (1489). — A full grown Japanese waltzing mouse.* This mouse presented a rounded mass, 1.5 centimeters in diameter, hanging from the neck just anterior to the fore-legs. The mass increased somewhat in size in the short period for which it was under observation. The mouse died from the shock of an operation for the removal of the mass. The gross appearance of the mass is as follows: It is covered with skin which is somewhat reddened and devoid of hair, but there is no ulceration. It is freely movable, and has a rounded slightly nodular surface. The tissue of which it is composed is light pink, rather soft, and, in addition to numerous minute cysts in its substance, there is a large cavity in its interior filled with a grayish, milky fluid.

The adjacent right axillary lymph nodes are somewhat enlarged, and there is a whitish line of tissue extending posteriorly into the mammary gland, and just posterior to this is a minute translucent nodule not over a millimeter in diameter. At the site of the left inguinal lymph node there is a small, sharply circumscribed nodule measuring 3 x 2 x 2 millimeters. In the right horn of the uterus is a minute translucent area about the size of a pin head. In the superior surface of the liver are many minute yellowish points.

The tumor consists of epithelium some of which is distinctly glandular in its arrangement. The lumina of the glands usually contain an eosin-staining, colloid material. Scattered through the tumor are vacuoles suggesting the presence of fat. Many of the tumor epithelial cells are filled with small vacuoles and such cells occur in nests, so that the structure of the sebaceous glands are closely simulated. In portions of the tumor the epithelial cells are flattened and spindle-shaped, so that they resemble the cells of a sarcoma. There is very little connective tissue in the structure of the tumor, but this is in places intimately intermingled with the epithelium. In certain areas the stroma presents large numbers of compound granule cells, with a peculiar form of degeneration. The nuclei appear hollow and the chromatin is arranged about the nuclear membrane. In the clear space within the nucleus is usually one or more masses of eosin-staining, hyaline material. In the stroma and surrounding tissue there are also small numbers of plasma cells, which are also markedly degenerated. The silver impregnation method of Levaditti brings out small numbers of spirochetæ, confined chiefly to the hyaline secretion and the cysts filled with broken down material. Mitotic figures are found in considerable numbers, but are not so numerous as in the inoculable mouse tumors.

The adjacent axillary lymph nodes are not invaded with this tumor.

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* Presented by Dr. Ernest Leroy Hunt, of Worcester, Mass.

The nodule of the left inguinal region consists of tumor structure similar to that just described, and it undoubtedly represents a metastasis of the latter. In the center of this nodule there is a compact mass of tumor surrounded by a thin capsule of connective tissue. Outside of this central mass the growth of the tumor is less dense and presents many small cystic cavities. This portion of the nodule presents a striking resemblance to the preputial gland. In this region the stroma presents large numbers of compound granule cells, many plasma cells, and relatively few eosinophiles and polynuclear leucocytes.

There is a small amount of hyaline material about the hair follicles, against the lower surface of the epidermis, and about the mammary glands. With the different stains, the hyaline material is colored similar to connective tissue.

The lung presents no pathological process.

The spleen contains a large amount of blood and considerable golden yellow pigment, situated within phagocytic cells. It contains many erythroblasts and developing polynuclear leucocytes. There is, in places, considerable disintegration of cells, especially of lymphoid and plasma cells. Many of the plasma cells are vacuolated, and many are filled with large hyaline globules. The lymph nodes contain well defined germinative centers, but, apart from these they appear to consist chiefly of plasma cells in various stages of degeneration.

The wall of the uterus contains a collection of cysts lined with cuboidal epithelium differing somewhat from the epithelium of the endometrium. These cysts are situated partly within and partly external to the internal layer of smooth muscle. In other portions of the uterus there are small collections of lymphoid and plasma cells, and large phagocytic cells with golden yellow pigment.

The primary adeno-carcinoma of the neck was inoculated subcutaneously into four normal Japanese waltzing mice. One of these was killed accidentally nine days after inoculation. The injected material, in this case, is represented by a mass measuring 3 x 5 millimeters, and consists of epithelium intermingled with connective tissue and newly-formed blood vessels without any definite structural arrangement. Mitoses were present in about the same frequency as they occurred in the original tumor. Within this mass is one large and several smaller collections of necrotic tissue which takes for the most part a pinkish stain.

A second mouse died forty-five days after inoculation. Beneath the skin there was a group of small, rounded, translucent nodules, the largest of which was about three millimeters in diameter. Portions of these nodules are glandular in structure and resemble the original tumor. Groups of vacuolated epithelial cells, resembling the sebaceous glands, are also found. In one portion many cells of the tumor epithelium appear small and compressed, and there is here and there evidence of degeneration.

In the third mouse the injected tumor material increased slightly in size, but since then has remained practically stationary for six and a half months subsequent to the inoculation and is now under observation.

In the fourth mouse the injected tumor material increased slowly, but eventually produced a group of nodules of considerable size. A histological study showed a tumor epithelium growing in the lymphatics at some distance from the larger nodules. After growing for three and a half months this tumor was successfully inoculated into other Japanese mice. Several common mice similarly inoculated furnished negative results.

The adeno-carcinoma of this case is of a peculiar type, in that groups of the epithelial cells become vacuolated so that the structure of the sebaceous and preputial glands is closely simulated. Fat is found in considerable quantity in these portions of the tumor, and also as numerous minute globules in portions where the epithelial cells are compressed and spindle-shaped.

The epithelium also presents a true glandular arrangement, and the lumen of the gland is usually filled with homogeneous, hyaline or colloid material. In portions of the tumor the epithelial cells are compressed and spindle-shaped, and intermingled with the connective tissue. Mitotic figures are not rare, but are not so numerous as in the Jensen tumor. The adjacent lymph nodes are not invaded, but the inguinal node is replaced by a tumor nodule similar in every respect to the primary tumor.

The predominating cells in the lymph nodes are cells of the plasma-cell type, and these show various degrees of degeneration.

The wall of one horn of the uterus contains a group of cysts (Fig. 12). It is impossible to determine whether this represents a congenital anomaly or a more recent development. The epithelium differs from that of the endometrium. Mitotic figures are not infrequent.

The inoculation of the adeno-carcinoma of this case into four normal waltzing mice has resulted in a slow but well defined growth in two, and further inoculations have been successful. One was killed accidentally nine days after inoculation. In the other, which died forty-five days after inoculation, the tumor epithelium was alive.

This tumor resembles very closely the one found by Loeb[7] in a Japanese waltzing mouse. He succeeded in inoculating other mice with this tumor, but certain of the tumors resulting from these inoculations were of a mixed type consisting in part of epithelium, in part of sarcoma tissue. The results obtained were therefore similar to those of Ehrlich,[3, 4] who by the inoculation of epithelial tumors produced at first mixed tumors containing both epithelial and sarcoma tissue, but which, on further inoculations, furnished a certain number of pure sarcomata in which there remained no epithelial tissue. Both Ehrlich and Loeb believed that the sarcoma element was not derived from the original primary tumor, but arose from the proliferation of the connective tissue of the inoculated animals.

Case No. 15 (1559). — A brown female mouse, exact age unknown, but evidently quite old. Four and a half months after its purchase a hard nodular mass was discovered behind left fore-leg. It was killed with chloroform and an autopsy made at once.

The skin is thin and tears readily. A mass situated in the mammary tissue behind the left fore-leg measures 1.4 x 1 x .5 centimeters. This is for the most part slightly translucent white in color, but on section more opaque grayish streaks and markings are apparent within it. It is hard and elastic — somewhat of the consistency of cartilage. Posteriorly this mass thins out, but it is otherwise rounded. By frozen sections it is found to be an epithelial tumor. A smaller mass of tumor tissue is situated just anterior to that already described, and another small mass is situated beneath the pectoral muscles. Both of these masses represent lymph nodes replaced by tumor. In the posterior surface of the superior lobe of the left lung is a white nodule about two millimeters across, and somewhat nearer the apex is a small adherent area. The cortex of the bones is much thicker than normal. The long bones such as those of the legs are hard, and the marrow-space is no thicker than a very fine cambric needle. The spleen is large and of dull, light red color. The other organs present no macroscopic lesions.

The mammary tumor was inoculated subcutaneously into eight normal mice. Hard nodules appeared in two of the mice inoculated, and increased steadily in size. In a third mouse there is a small mass which has not as yet shown any appreciable growth. Sufficient time has not elapsed to determine the results of further inoculation.

In the skin of this mouse the hair follicles and sebaceous glands are surrounded by faintly-staining, hyaline material, staining blue with Mallory's connective tissue stain. In places the material is found lying against the inner surface of the epidermis, and it surrounds nearly all the blood vessels of the skin, capillaries included. In places it appears as a continuous layer of hyaline, in others it has the form of very thick wavy fibers. The mammary glands are, to a large extent, replaced by this hyaline material, and are represented by irregular branching cords of hyaline in which there is a core of crowded epithelial cells intermingled with which is yellowish pigment.

The tumor is composed of epithelium, the arrangement of which is peculiar in that in it are everywhere found irregular clefts. From this it is apparent that there is a tendency on the part of the tumor epithelium to irregular papillary ingrowth.

The stroma is small in amount, but is apparently dense. A large portion of the primary growth is necrotic, and the living and dead tissue is intimately intermingled. The epithelial cells are somewhat smaller than those of the Jensen tumor, and the cell-outline is indistinct. The nuclei are vesicular, and, as they are relatively large, appear closely packed in the tumor epithelium. Mitotic figures are everywhere numerous. The epithelium, although in places it occurs in rather large solid masses, tends usually to form surfaces within its substance. It is seldom that the surfaces bound cylindrical spaces as is the case with the lumina of glands, but the surfaces almost invariably bound very irregular clefts in the epithelium, so that it has an irregular papillary arrangement. The tumor has infiltrated the overlying skin and comes into contact with the epidermis. There is a small ulceration at one point. The fibers of the panniculus carnosus involved in the tumor present various degrees of degeneration. The tumor epithelium has grown along a large nerve which is situated beneath it, and is also found in a subcutaneous lymphatic at a distance from the border of the tumor.

The mass situated just anterior to the primary tumor consists of tumor tissue which has almost completely replaced a lymph node. The peripheral sinus is outlined by a layer of tumor epithelium, and there is a small amount of lymphoid tissue in one portion of the nodule. The central portion of the mass presents a large amount of necrotic tumor material. On one side, the tumor has infiltrated the connective tissue. The structure of the tumor is here identical in every respect with that of the primary growth. Relative to the amount of necrosis present there is but little inflammatory reaction.

The next lymph node situated beneath the pectoral muscle presents a more recent involvement with the tumor. Portions of its structure still remain and the follicles are readily distinguished. The peripheral sinus is on one side filled with a solid mold of tumor epithelium, but there is at one point an accumulation of lymph, slightly dilating the capsule of the lymph node. There are also other smaller dilatations of the lymphatics evidently due to obstruction by the masses of tumor epithelium. As compared with the primary growth, there is here a relatively small amount of necrotic tissue.

The nodule in the lung is a tumor of another type from that of the mammary gland. It consists of cuboidal or columnar epithelium growing upon irregular folds or septa of connective tissue. The cytoplasm is much more abundant in this epithelium than in the mammary tumor and it stains more intensely with eosin. Several large blood vessels extend into its substance. Whereas the mammary tumor presented large numbers of mitotic figures, the epithelium of this tumor presents none.

The nuclei are often irregular and many contain peculiar hyaline inclusions such as have been found in other tumors of this type situated in the lung. Direct nuclear division is apparently common. Near the periphery of the nodule are several small collections of lymphoid cells. Other portions of the lung appear practically normal. At one point on the pleura there is a small mass of inflammatory tissue, probably of the nature of an adhesion.

In the liver, about the portal canals, are large collections of cells, most of which are of the leucocyte series. Intermingled with these are large cells filled with yellow pigment, with here and there delicate needle-shaped crystals. In the kidney there are numerous minute foci of acute interstitial nephritis. Many of the tubules contain casts. There is a moderately severe infection with Klossiella muris. Between the cortex and medulla of the adrenal there are several large groups of cells with small shrunken nuclei and abundant cytoplasm, which is filled with coarse irregular granules, and is somewhat vacuolated. The cells have a slightly greenish tinge and are probably phagocytes filled with some form of pigment.

The spleen is large and active, erythrocytogenesis and leucocytogenesis are marked. These processes are more prominent than is the formation of lymphoid cells. In certain areas there are cells loaded with pigment. There is no amyloid and nowhere any destructive process.

The lymph nodes, other than those invaded by tumor, are not notably enlarged, but present considerable activity. Besides the formation of cells of the lymphoid series there is, in all those studied, evidence of more or less leucocytogenesis. In many instances the sinuses are filled with large phagocytic cells, in the cytoplasm of which are found large numbers of slender needle-shaped crystals which stain with eosin. These cells also contain more or less yellowish pigment and occasionally portions of other cells. There is considerable disintegration of cells of the lymphoid and plasma-cell type.

The bone-marrow of the femur is reduced in amount. Its activity appears to be chiefly leucocytogenic, and erythroblasts are but rarely seen. There are many large cells with a pinkish-staining cytoplasm which contains masses of sharp-pointed, slender crystals, pigment, red blood corpuscles and leucocytes. The cortex of the bone is thick and stains unevenly.

The glands of the stomach are dilated and filled with large numbers of a unicellular organism, many of which are in the process of segmentation. They are invariably found in the secretion of the glands and never occur within the cells. Certain ones are encapsulated. Beyond the dilatation of the peptic glands there is no reaction on the part of the tissue.

Both large and small intestines appear normal.

In this case there occurred in an old mouse, which had never been inoculated, a primary adeno-carcinoma ("spaltenbildendes carcinom") of the mammary gland with metastasis to the adjacent lymph nodes, and also a primary papillary cyst-adenoma of the lung. The mammary tumor in the gross appeared whitish in color and was hard and elastic. It is composed of epithelium which is irregularly papillary in arrangement, so that it forms irregular surfaces, and contains many cleft-like spaces. It is actively growing, infiltrates the surrounding fat and connective tissue, and is found growing in the subcutaneous lymphatics. The stroma of the tumor is small in amount, but appears very dense. A large portion of the tumor is necrotic. This tumor has the same structure and arrangement where it has invaded the lymph nodes. The same tendency to undergo necrosis is here also apparent. The tumor of the lung is of another type and is identical with other primary tumors which have been found in the lungs of mice. It presents no mitotic figures.

In addition to primary tumors of two distinct types, this mouse presents many other pathological changes. The skin, which appeared rather thin and friable in the gross, shows a peculiar process. The small blood vessels and capillaries of the skin are surrounded by faintly-staining, hyaline material. This is also deposited about the sebaceous glands and hair follicles and is found in places beneath the epidermis. This material has to a large extent replaced the mammary gland. The ramifications of the gland are outlined in hyaline, but there is usually found a central core of degenerating gland-epithelium, intermingled with which are small collections of yellow pigment.

The lymph nodes, liver, and spleen show marked leucocytogenic activity. The bone-marrow is much reduced by a general osteosclerosis, but other than the presence of phagocytic cells filled with needle-shaped crystals, it shows no important pathological process. The lymph nodes and spleen also contain large numbers of phagocytic cells filled with masses of slender, needle-shaped crystals. The kidney of this mouse shows, besides a slight nephritis, a severe infection with Klossiella muris. Another species of parasitic protozoan is found in large numbers in the dilated glands of the stomach.

Case No. 16 (1647). — A white female mouse of unknown age and never inoculated. Six and a half months after purchase a rounded, somewhat triangular mass was observed just anterior to right hind leg and extending upwards toward the back. This mass was removed by operation, and was found to be in close relation to the mammary gland at its lower angle. The mass measures 1.8 x 1 x .8 centimeters, and has an outer convex, slightly nodular, surface and a concave, smooth, inner surface where it lies against the body wall. In its outer surface are rounded blackish and dull reddish areas, some of which are very minute, others a millimeter or more in diameter. The consistency of the tumor is moderately firm, but its tissue is easily crushed into a homogeneous mush. It appears to be subdivided into rather coarse lobules. A portion of the tumor was fixed for histological study, and the remainder inoculated into ten normal mice. Nothing has developed as the result of these inoculations.

The tumor consists of epithelium showing everywhere a tendency to glandular arrangement. The dark coloration of certain portions of the tumor are due to hemorrhage into its substance. The tumor is subdivided by septa of connective tissue into lobules, many of which are relatively large. Different portions of the tumor vary greatly in structure. Certain lobules are cystic, and there is in some instances papillary ingrowth of the epithelium. In other portions dense connective tissue has apparently grown into the glandular tissue, and has replaced it to a large extent. In other portions of the tumor the glands are filled with dense colloid material which takes the eosin stain. In the large lobules of the tumor the epithelium has a distinct glandular arrangement, but there is very little connective tissue, and elastic tissue is practically absent within the lobules. The blood vessels are here of large size, but extremely thin walled. There are many large spaces in the tumor epithelium, and these spaces are often traversed by the thin walled blood vessels, and it is here that hemorrhage apparently occurs, being dependent upon the delicacy of the vessel walls and the looseness of the surrounding tissue. In the older hemorrhages the red blood corpuscles are to a large extent disintegrated, and their substance is being taken up by phagocytic cells. In places there are large numbers of pigmented cells in the connective tissue around such a hemorrhage. In the connective tissue there are, in places, collections of lymphoid and plasma cells and numerous eosinophiles. There are also many nongranular leucocytes. Mitotic figures are present in the tumor epithelium in moderate numbers.

The mouse in which this tumor developed recovered from the operation and remained well and vigorous for nearly two months after the operation. There was no evidence during life of recurrence at the site of operation. Following pregnancy the mouse appeared ill and the abdomen became asymetrically distended. On killing the mouse the distention is found to be due to a large collection of pus and necrotic material enclosed in a portion of one of the horns of the uterus. The abscess cavity measures 2.5 centimeters in diameter and contains a macerated fetus. All the other organs of the peritoneal cavity are bound up in adhesions. The liver appears larger than normal and has a dull brownish color. Spleen large, rounded, and dark red. The lower lobe of the right lung presents a translucent, whitish nodule three millimeters in diameter. There is no trace of the operation wound in the inguinal region, but near by there is a small nodule 2.5 millimeters in length.

The nodule which occurred near the site of operation in the inguinal region is similar histologically to certain portions of the original tumor previously excised. It is glandular in structure throughout, and presents considerable colloid material within the lumina of the glands. The nodule in the lung possesses a structure identical with that of the primary tumors of the lung already described. It contains a cyst about 1.5 millimeters in diameter. There are numerous eosin-staining bodies found in the cytoplasm of the tumor cells. Many of the nuclei also present eosin-staining hyaline bodies. Mitotic figures, many of which are atypical, are found in this tumor.

The capillaries of the lung contain great numbers of polynuclear leucocytes. The lungs also present a peculiar hyaline thickening of the intima of the pulmonary veins. The liver and spleen, as well as the bone marrow, show marked leucocytogenic activity.

In this mouse there are two primary tumors, one arising in the inguinal region, the other in the lung. The former is of the type described by Apolant as Cysto-carcinoma hemorrhagicum. Different portions of this tumor vary greatly in structure. Large portions of the tumor possess very little supporting tissue. The structure of the tumor is loose, and there are spaces within the lobules filled with fluid and in some cases possessing a very delicate reticulum. The blood vessels ramify through these spaces, and, since they are thin walled, hemorrhage readily takes place. Portions of this tumor present a more characteristic glandular structure and there is considerable eosin-staining, colloid material within the gland alveoli. In portions of the growth, where there is a peculiar intermingling of connective tissue with the glands, it appears that spaces occurring in loosely constructed epithelial tissue have become filled with proliferating connective tissue. Following the removal of this tumor recurrence took place, a small nodule two and a half millimeters in length being found two months after the operation.

The primary tumor of the lung is similar in structure to those already described in the preceding series. It is peculiar in that it presents mitotic figures in moderate numbers. These had, heretofore, never been observed in the primary tumors of the lung.

This mouse appeared well and vigorous for two months subsequent to the removal of the inguinal tumor, and became pregnant. It was killed on account of a septic condition complicating pregnancy.

Considering the various types of spontaneous tumors which have thus far developed, the papillary cyst-adenomata of the lung have been by far the most frequent in occurrence, although tumors of this type seem to have attracted but slight attention. These tumors, on account of their situation, are only found by careful post-mortem examinations and some owing to their minuteness may, even at that, be overlooked. They occur in males as well as females, often in individuals which are not of great age. Since mitotic figures have been found in only one of them, it is probable that their growth is usually relatively slow. On account of the nature of the organ in which they develop, they may be malignant. The lung may be almost entirely replaced by a tumor of this type. The tumor enlarges not only by internal expansion, but its epithelium also grows outward into the lung along the alveolar walls and sometimes grows into the bronchi. In this series all the tumors conform to one general type of structure, although there are minor variations even in different parts of the same tumor.

The cyst-adenoma occurring in the kidney also appears to be a slow-growing tumor. It seems probable that such growths originate by the irregular proliferation of the epithelium of cysts within the kidney. The tumor epithelium resembles somewhat that of the kidney tubules.

A group of cysts found in the uterus of one case may possibly represent a congenital anomaly, although this cannot be determined.

The lympho-sarcomata of this series are actively-growing tumors, and consist chiefly of cells similar to those found in the germinative centers of lymph follicles. The blood vessels present blind branches and spurs such as are found in the developing blood vessels of granulation tissue. Large phagocytic cells are found in certain portions of the tumor.

The four subcutaneous adeno-carcinomata are all of different types. In one case the primary tumor presents all the transitions between a simple adenoma and an active, irregularly growing, carcinoma. The tumor of the second case is peculiar in that groups of its epithelial cells appear vacuolated so that the structure of the sebaceous follicles is closely simulated. Other portions have a true glandular arrangement. In the third case the primary tumor is hard and elastic. The hardness of this tumor appears to be due to the great density rather than to the amount of its stroma. It is composed of epithelium having an irregular papillary arrangement so that there are irregular or crescentic clefts. There is a relatively large amount of necrosis. The fourth tumor, on account of its loose structure and the tendency of the epithelium to form spaces, shows a marked degree of hemorrhage.

As might be expected the tendency to metastasize is much greater in certain tumors than in others. Multiple nodules were found in certain of the cases of papillary cyst-adenoma of the lung, but it could not be determined whether these originated by transplantation of the tumor epithelium by the way of the air passages, or whether they represented multiple primary growths. In the cases of adenoma of the kidney there was no metastasis. In one of the cases of lympho-sarcoma there was no metastasis, but in the other there were metastases in the pericardium, pleura, and lungs. With three of the four subcutaneous adeno-carcinomata there was invasion of the lymphatic system. With the mammary tumor, which showed transition from a simple adenoma to a carcinoma, what was considered to be the metastasis was of a type somewhat unlike the primary growth. In the other two cases the tumor-tissue replacing the lymph nodes retained the structural arrangement of the primary growth.

There appears to be a difference between the spontaneous tumors and the Jensen and Ehrlich "Stamm 11" inoculable tumors, in that the former invade the lymphatic system whereas the latter similarly situated have never done so, but have metastasized by way of the blood stream. However, in a successful inoculation with the tumor of a Japanese waltzing mouse, Case No. 14, tumor epithelium is found growing in lymph vessels at some distance from the main mass of the tumor. It would also appear that metastasis is more frequent in the spontaneous than in the inoculated tumors, at least when the latter are situated subcutaneously.

The results of the inoculation of normal mice with six of the tumors of this series are as follows:

| Tumor Inoculated. | No. of Inoculations. | No. of Results. | Positive Results. |
|---|---|---|---|
| No. 1. Cyst-adenoma of lung......... | 5 | 5 | 0 |
| No. 11. Lympho-sarcoma ............. | 17 | 17 | 0 |
| No. 13. Carcinoma simplex (metastasis). | 10 | 0 (Suppuration) | 0 |
| No. 14. Japanese carcinoma........... | 4 | 2 | 2 |
| No. 15. Papillary carcinoma........... | 8 | 7 | 2 |
| No. 16. Hemorrhagic carcinoma ....... | 10 | 10 | 0 |
| 6 tumors.......................... | 54 | 41 | 4 |

Thus only two of the six tumors inoculated have been successfully propagated, and these two were subcutaneous adeno-carcinoma. The number of inoculations presented is not sufficiently great to make the determination of the percentage of positive results of value. It is to be noted, however, that whereas certain tumors grow readily on transplantation, other tumors fail to grow even when inoculated under the most favorable conditions, so that the inoculable tumors undoubtedly possess possibilities of growth not shown by other tumors.

Inasmuch as the epithelium of the inoculable tumor is able to remain alive under unfavorable conditions, such as freezing and subjection to various chemicals, it appears that it is its relatively great vitality which enables it to grow on various soils such as are found in the tissues of different individuals. The noninoculable tumors, on the other hand, appear to require some special condition for their growth, a condition not found generally in other individuals.

The pathological processes found in relation with spontaneous tumors may possibly be of the nature of senile changes, or they may represent coincident conditions. Nearly all the lesions found in mice with tumors occur also in mice with no tumors. Parasites of various types are frequently met with. Bacteria are found in the lesions of various organs. Spirochetæ are sometimes found within the primary tumors as well as in other tissues. The significance of their occurrence will be taken up in another paper.

Sarco-cystis muris in the voluntary muscles and Klossiella muris in the kidney are of common occurrence. Encysted worms have been found in the lung and in the kidney. As marked, an atrophy of the liver was found in mice without tumors, as was found in Case No. 10, which presented an adenoma of the kidney. Nephritis is so frequent in the mouse that no significance can be ascribed to its occurrence in mice with tumors. Amyloid is frequently found in the liver, spleen, kidneys, and adrenals, and less frequently in the intestine in mice which are cachectic from bacterial infection. In several mice with tumors there was a deposit of hyaline around the small blood vessels, hair follicles, and occasionally just beneath the epithelium of the skin. In one case this material was deposited about the acini of the mammary gland, which had atrophied to such an extent as to be scarcely recognizable. In the same case there was also a general increase in the thickness of all the bones.

Tumors in mice may interfere with the body functions, especially if organs such as the lungs or heart are involved. In such instances the mere mechanical action of the tumor may cause death. On the other hand, certain mice possessing external primary tumors of great size appear strong and vigorous. Thus, since there is not evidence to establish any causal relation, it seems probable that the pathological processes found in mice with tumors represent coincident conditions.

Concerning the frequency of tumors among mice the consideration of numbers is of little significance unless age is considered as well. The sixteen cases here recorded occurred in somewhat less than eight hundred mice which have been under observation during two years past. Of these about five hundred either have been killed or have died, and the others are now under observation. An autopsy is made on each mouse which dies, in order to determine the presence of internal as well as external tumors. The best possible conditions are afforded the mice kept under observation so that they may attain as great age as possible. In certain instances old mice have been purposely purchased, and it is among these that two of the four subcutaneous tumors have occurred. The infrequency of tumors in mice indicated in the reports of certain investigators who have had enormous numbers of these animals under observation may be possibly explained in the failure to enable them to attain sufficiently great age.

In the present series of cases, the frequency of occurrence of double primary tumors in mice is to be noted. In sixteen mice with primary tumors there was a second primary tumor of a different type in four (or twenty-five per cent). This does not include the case which presented a collection of cysts in the uterus in addition to a subcutaneous tumor. Thus, in a given number of mice possessing a primary tumor, the probability of occurrence of second primary tumors appears to be greater than is the probability of occurrence of primary tumors in an equal number of mice, of the same age, taken at random.

The fact, stated earlier in this paper, that certain of the primary tumors of this series occurred in mice previously inoculated with tumor tissue is worthy of comment. The question at once arises, " Are these primary tumors to be regarded as the effects of the inoculations? " Ehrlich[3, 4] believes that, in certain instances, the inoculation of epithelial tumors into normal mice has in some way stimulated the connective tissue at the site of the inoculation to proliferate so that there resulted a mixed tumor, which on further transplantation resulted in a pure sarcoma. Loeb[7] also obtained in Japanese mice a growth of sarcoma by inoculating a carcinoma. Haaland[5] found nodules arising by the proliferation of the alveolar epithelium in the lung of a mouse which had been previously inoculated with the Jensen tumor. From this he suggests the possibility of metastasis of tumors without the actual transplantation of cells.

In twelve of the cases presented in this paper, primary, papillary cyst-adenomata of the lung were found in six uninoculated mice and also in six mice which had been previously inoculated with tumor tissue. In one of the latter the inoculation of Ehrlich's "Stamm 11" had resulted in a large actively-growing tumor; in another mouse a lympho-sarcoma, which had been inoculated, failed to grow as it did in all other cases; and in four mice, inoculated with the Jensen tumor, there was in one a slow-growing tumor, in one a mass which developed at the site of inoculation, but later disappeared spontaneously, and in two no growth at the site of inoculation. In one of the latter there was, in addition to the primary tumor of the lung, a primary growth in the inguinal region which appeared soon after the inoculation. In all of these cases it is possible that the primary tumors of the lung may have been present when the inoculations were made. It is of course impossible to determine this point on account of the situation of these tumors. In the case presenting the mammary tumor, two nodules, one representing the primary growth, the other the metastasis, were first noted five weeks after the inoculation. The short period intervening between the inoculation and the appearance of this tumor with its metastasis makes the hypothesis untenable that it developed as the result of the inoculation. Since in a long series of inoculations with the Jensen tumor there has been no similar instance, it seems reasonable to regard the occurrence of a primary tumor, following an inoculation, as an example of coincidence rather than cause and effect.

When inoculated and uninoculated mice are compared as regards the frequency of occurrence of primary tumors, it is of interest to note that, in those kept under observation, primary tumors are more frequent in the uninoculated. This is possibly explained by the fact that a large number of the inoculated mice die at a comparatively early age as the result of the tumors which develop, and this number probably includes those mice which are most susceptible to tumor growth. Only those in which the inoculation results negatively live to attain old age, and many of them must be regarded as immune to tumor growth.

## SUMMARY.

1. Papillary cyst-adenomata of the lung have been found in twelve mice. These tumors consist of epithelium covering thin folds and processes of supporting tissue in which there is a relatively large amount of elastic tissue. Since mitotic figures were found in only one tumor of this type, they are probably of slow growth, but may attain such size as to interfere with the function of the lung, and sometimes grow into the bronchi.

2. Cyst-adenomata of the kidney such as were found in two cases probably arise by the irregular growth of the epithelium lining the cysts. They show no evidence of active growth, and are probably benign.

3. Two lympho-sarcomata, one arising in the inguinal region of a female, the other in the mediastinum of a male

mouse, consist chiefly of cells resembling those found in the germinative centers of the lymph follicles. They contain little supporting tissue, but are permeated with small actively-growing blood vessels. Phagocytic cells are numerous in some portions of these tumors. These tumors appear to be of the nature of definitely localized primary growths. In one case, other portions of the body were not involved, in the other there were metastases in the pericardium, pleura, and lungs.

4. Subcutaneous adeno-carcinomata occurring in four female mice, are all of different types. Metastasis had occurred in three of these cases. In the fourth animal recurrence followed the excision of the primary tumor.

The tumor in the first case presents an irregularly growing carcinoma originating in an adenoma of the mammary gland (Carcinoma simplex alveolare). The tumor nodule is sharply defined and there is no evidence of transition from normal gland to adenoma. Transitions between the glandular and the irregularly-growing carcinomatous tissue are, however, apparent in certain portions.

An adeno-carcinoma which developed on the neck of a Japanese waltzing mouse is of a peculiar type. Many of the epithelial cells contain fat, and there are nests of large vacuolated cells, so that the structure of the sebaceous follicles and preputial glands is closely simulated. In portions of the tumor the epithelium is intimately intermingled with the connective tissue. Here the epithelial cells are compressed and spindle-shaped.

The third subcutaneous adeno-carcinoma presents everywhere irregular narrow fissures in the epithelium ("Spaltenbildendes carcinom"). The unequal growth of the glandular epithelium has resulted in a tumor irregularly papillary in type. The epithelium is found growing in the lymphatics.

The fourth subcutaneous adeno-carcinoma, which recurred after excision, presents various types of epithelial growth and shows a marked tendency to hemorrhage (Cystocarcinoma hemorrhagicum). The glandular portions of this tumor contain colloid material.

5. Six of this series of spontaneous tumors have been inoculated into normal mice. Of four subcutaneous adenocarcinomata, two grew on transplantation, one failed to grow, possibly by reason of an accompanying infection, and with the fourth the results of the transplantation were negative. The inoculation of a rapidly-growing lympho-sarcoma into normal mice resulted negatively. Inoculations with a papillary cyst-adenoma of the lung furnished only negative results.

6. Spontaneous tumors originating subcutaneously appear to metastasize much more frequently than either Jensen's or Ehrlich's "Stamm 11" inoculable tumors similarly situated. The lymph nodes are frequently invaded by the spontaneous tumors, but this has never occurred with the above inoculable tumors, in which metastasis always takes place by way of the blood vessels.

7. An inoculable tumor in a Japanese mouse differs from the Jensen and Ehrlich "Stamm 11" tumors in that it grows into the lymphatics.

8. The papillary cyst-adenomata of the lung and the lympho-sarcomata were found in both males and females. The other types of tumors were found only in females.

9. No special significance is found in the pathological processes accompanying tumors in mice. In many instances such conditions as result from infections with a variety of microörganisms, or possibly from old age, are regarded as coincident with but not immediately related to the development of the tumors.

10. The occurrence of primary tumors in mice previously inoculated is not sufficiently frequent to be regarded other than as instances of coincidence.

11. Double primary tumors are of frequent occurrence.

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## DESCRIPTION OF PLATES.*

### PLATE VII.

FIG. 1. — A minute primary tumor of the lung in a mouse, Case No. 9. There is a large blood vessel extending through the central portion of the tumor.

FIG. 2. — A papillary cyst-adenoma of the lung, Case No. 1. Lung tissue is present at the sides of the tumor mass. The surface of the tumor is slightly depressed over its central portion where it has been replaced by connective tissue.

FIG. 3. — A small papillary tumor of the lung, Case No. 3. At the lower right hand border of the nodule is a collection of cells of a type distinct from those of which the tumor is composed. They are probably desquamated alveolar epithelial cells.

### PLATE VIII.

FIG. 4. — A higher magnification of the tumor represented in Fig. 2, showing the epithelium covering the folds and processes of connective tissue.

FIG. 5. — The periphery of the same tumor, Case No. 1. The tumor is apparent in the right hand portion of the photograph. Individual tumor epithelial cells are to be seen here and there on the alveolar walls. The alveoli are filled with cells of a type distinct from the tumor epithelium.

FIG. 6. — A primary cyst-adenoma of the lung, Case No. 2, stained with Weigert's elastic tissue stain. There is an increase in the amount of elastic tissue in that portion of the pleura covering the tumor. The tumor contains a larger amount of this element than does the normal lung tissue.

FIG. 7. — A hyaline body situated within a nucleus of an epithelial cell of a tumor of the lung. These are frequent in tumors of this type in mice, but occur in other tissues as well.

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* I am indebted to Dr. S. B. Wolbach, Instructor in Pathology, Harvard Medical School, for aid in illustrating this article.

Fig. 8. — A papillary cyst-adenoma of the lung, Case No. 8. The tumor has grown into a small bronchus where it is distinctly papillary in type.

## Plate IX.

Fig. 9. — A cyst-adenoma of the kidney, Case No. 10. The nodule consists of a mass of epithelial tissue invaginating a cyst. The nodule is distinct from the kidney tissue below.

Fig. 10. — A cyst of the kidney with irregular proliferation of its lining epithelium, showing the possible mode of origin of certain forms of cyst-adenomata in the kidney.

Fig. 11. — Cyst-adenoma of the kidney, occurring in Case No. 1. The kidney tissue occupies the lower left hand portion and the adenoma the upper right hand portion of the field.

Fig. 12. — Cysts occupying the wall of the uterus, Case No. 14, in which there is also a subcutaneous adeno-carcinoma.

## Plate X.

Fig. 13. — A subcutaneous adeno-carcinoma, occurring in an old female mouse, Case No. 13, inoculated several weeks previous with the Jensen tumor. Near the border of the tumor a portion of the mammary gland is apparent. The peripheral portion of the growth is purely glandular, but in its central portion the arrangement of the epithelium is very irregular and there is evidence of active growth.

Fig. 14. — A lympho-sarcoma which appeared in the inguinal region of an old female mouse, Case No. 11. The tissue is of the type found in the germinative centers of lymph follicles. The cell outlines are hardly distinguishable. x 1,000 diameters.

Fig. 15. — A portion of the subcutaneous adeno-carcinoma of a Japanese waltzing mouse, Case No. 14. Within the lobules groups of cells become fatty and break down to form spaces so that structures are formed similar to the sebaceous follicles.

Fig. 16. — A portion of the metastasis of the adeno-carcinoma of Case No. 13, shown in Fig. 13. Near the center are structures having somewhat the character of epithelial pearls.

## Plate XI.

Fig. 17. — A subcutaneous adeno-carcinoma in a Japanese waltzing mouse, Case No. 14. The tumor is peculiar in that groups of cells contain fat so that structures occur which resemble very closely the sebaceous follicles of the skin. The epithelium and connective tissue are intimately intermingled, and the epithelial cells are often spindle-shaped. The stroma of the tumor is quite cellular and there are many compound granule and plasma cells.

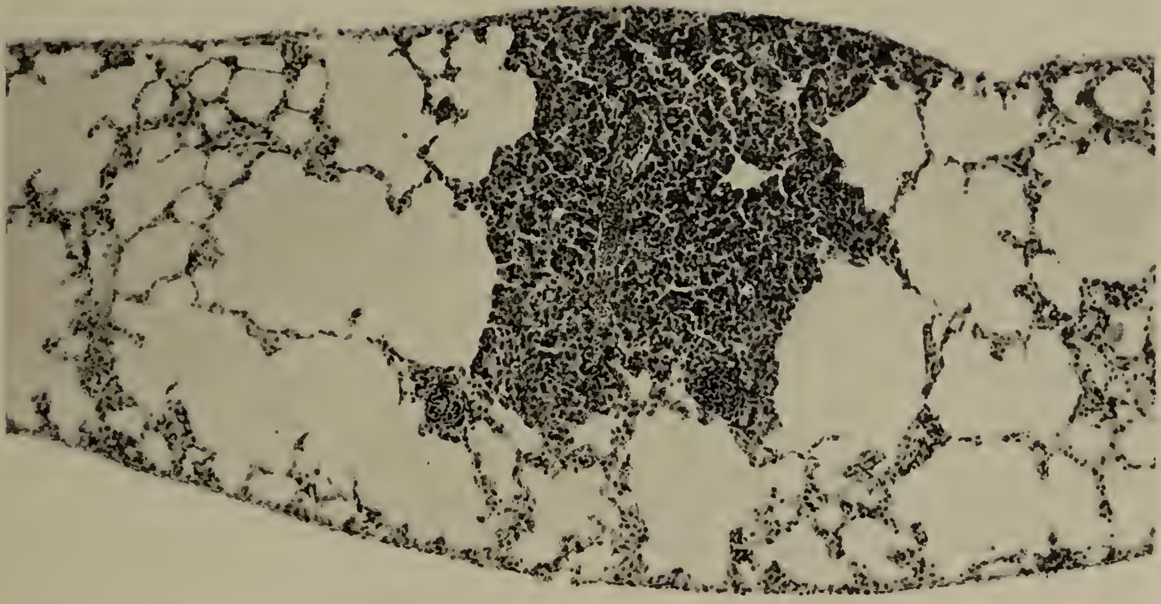
Fig. 18. — A tumor nodule interpreted as a metastasis of the tumor shown in the figure preceding. This was found in the inguinal region,

whereas the larger tumor was found in the neck. The structure of the preputial gland of the mouse is clearly simulated in this smaller nodule.

### PLATE XII.

FIG. 19. — A papillary adeno-carcinoma of subcutaneous origin, Case No. 15. The primary tumor as well as the metastasis shows a constant structural peculiarity in the formation of irregular clefts in the epithelium.

FIGS. 20, 21, 22. — Cysto-carcinoma hemorrhagicum, Case No. 16. Photographs taken from different portions of the tumor to show different types of structure. In the first figure the vascularity of the tumor combined with the tendency of its epithelium to form spaces is indicated. The portion of tumor next shown is glandular in structure, and contains colloid material. In the last figure there appears to be great proliferation in the connective tissue, which is invading the tumor.



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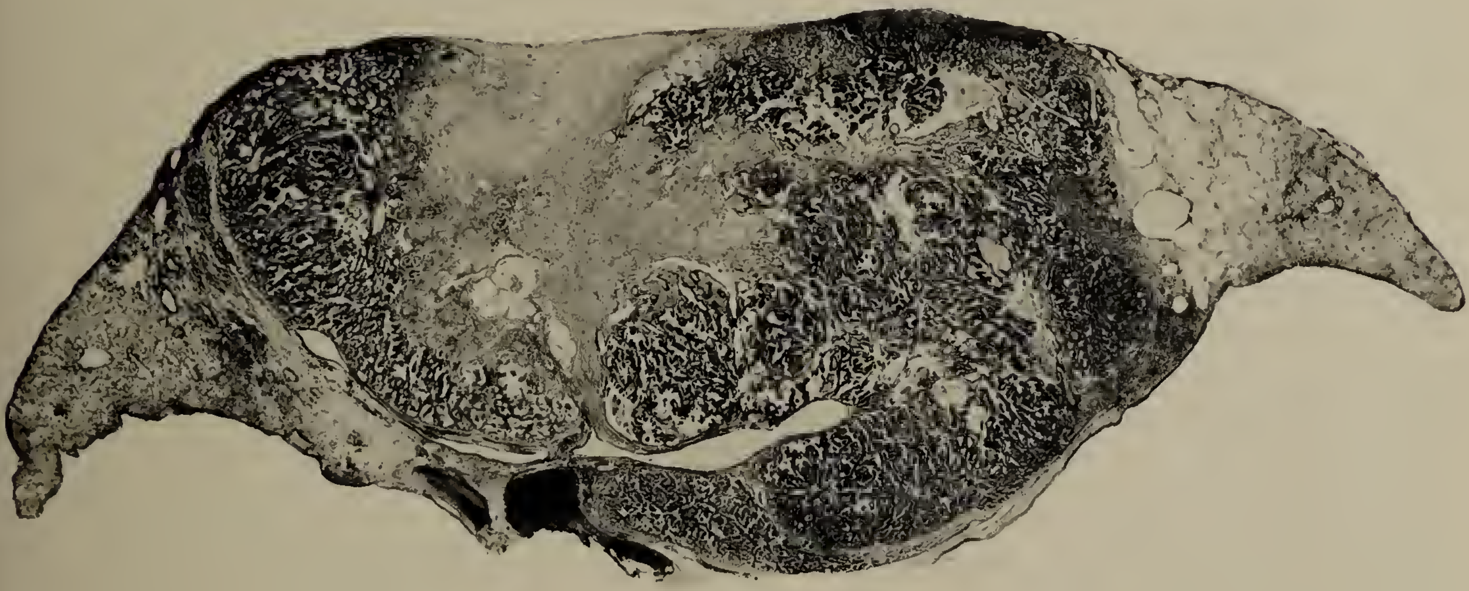

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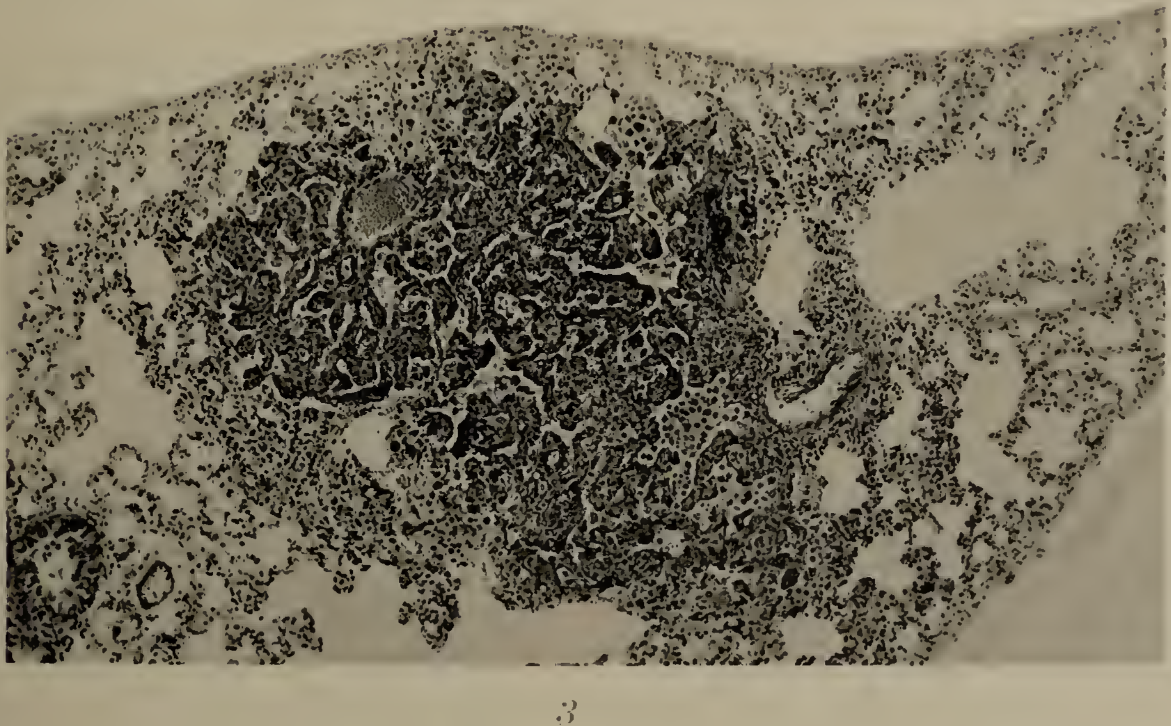

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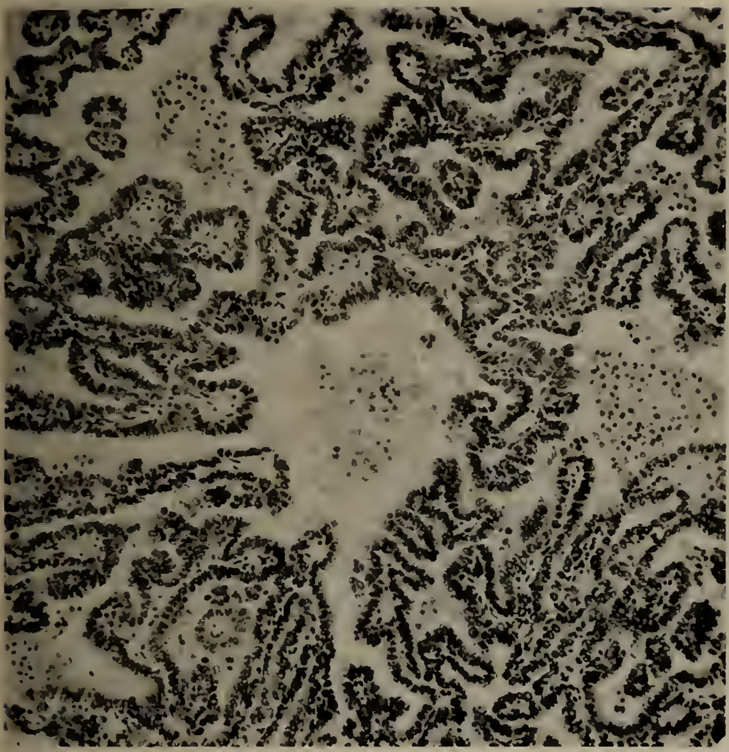

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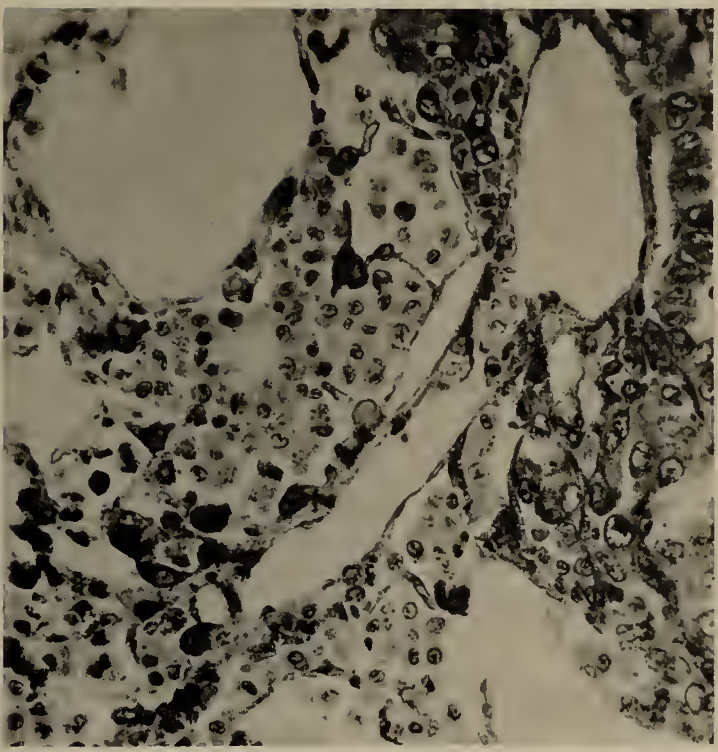

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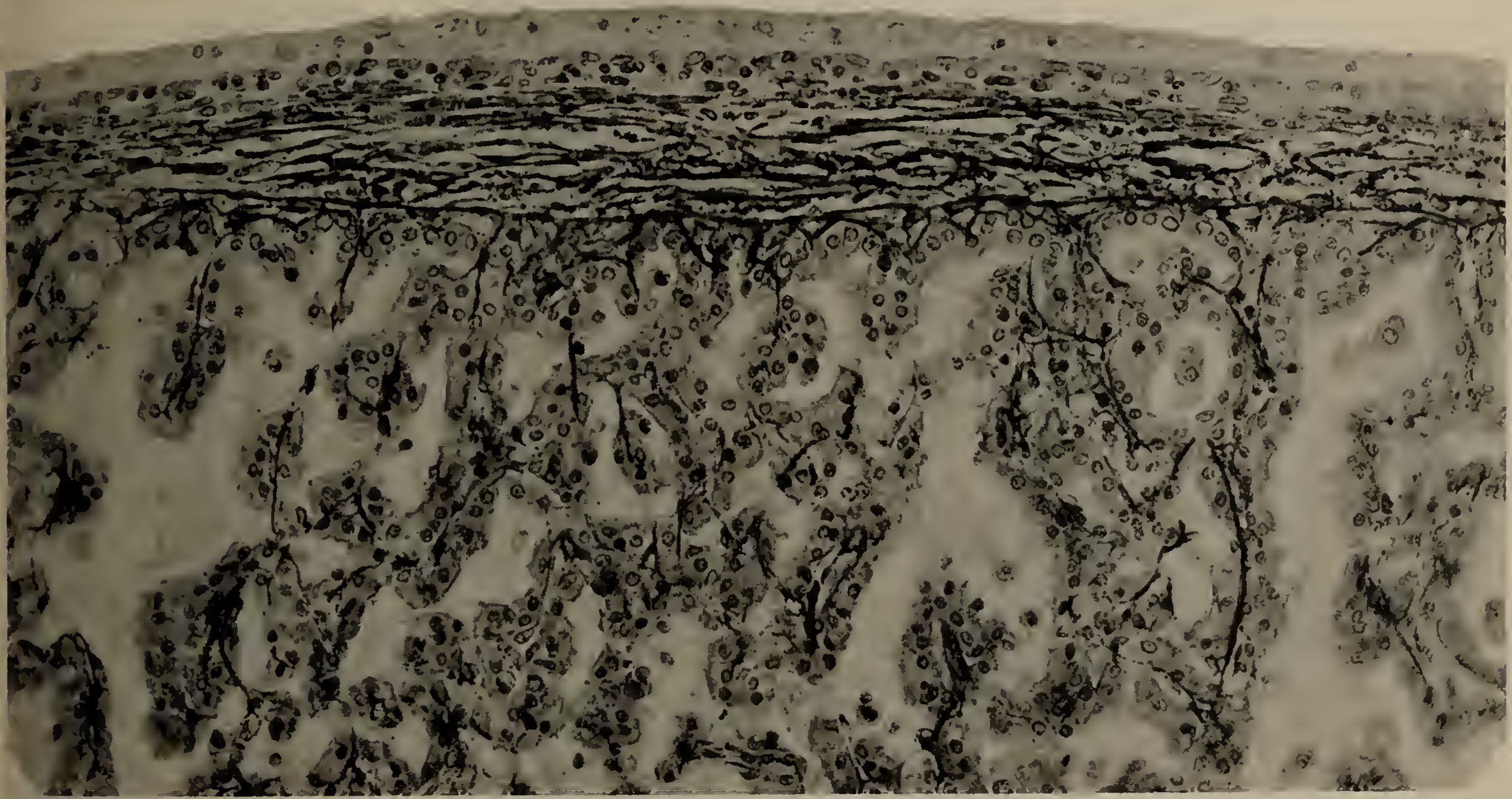

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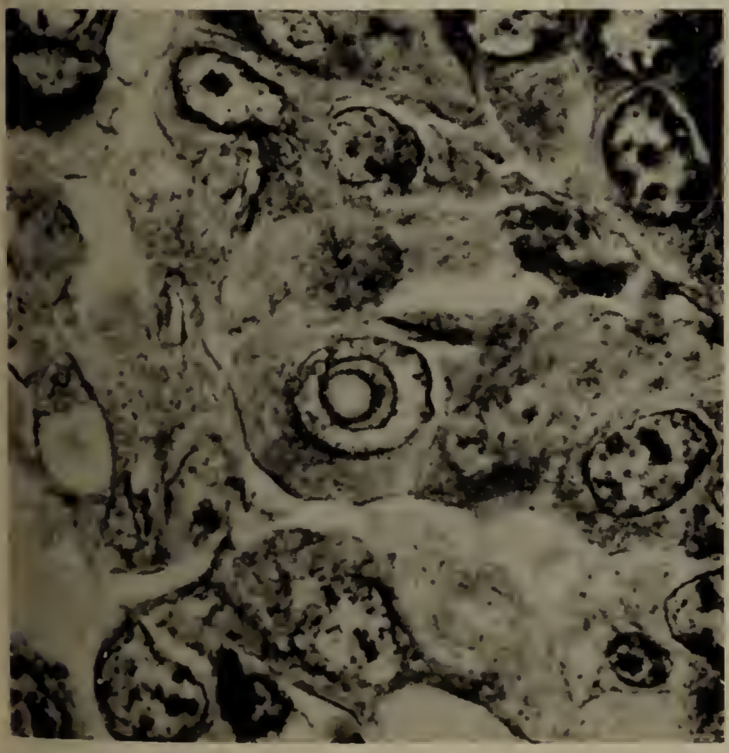

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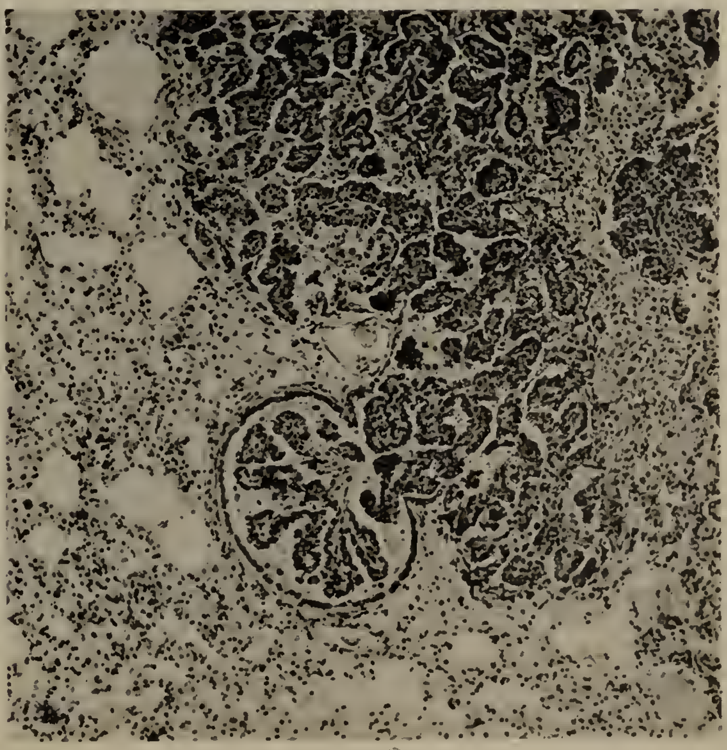

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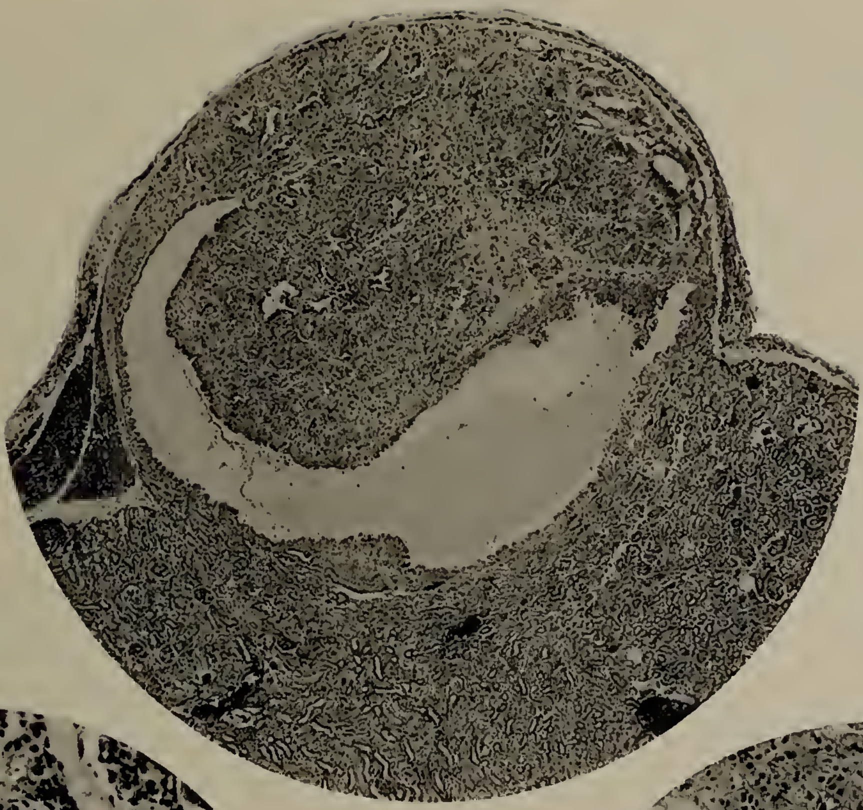

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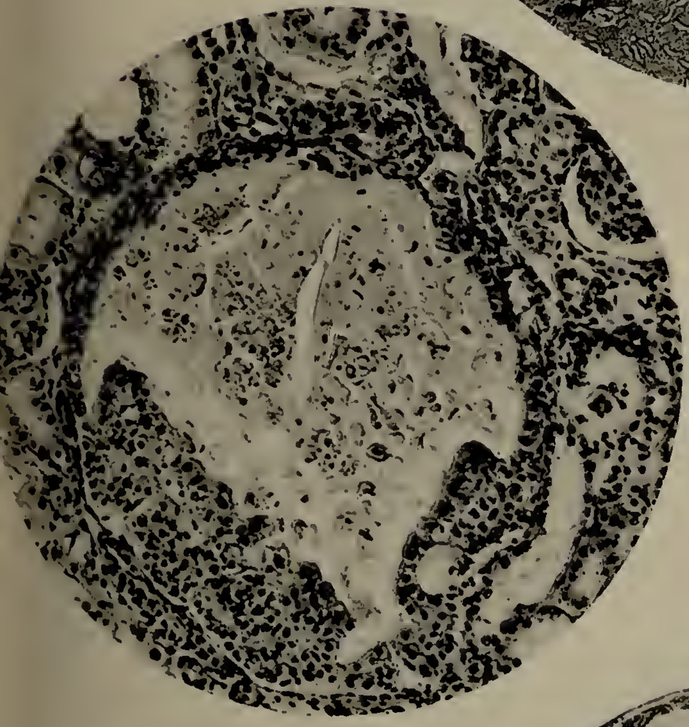

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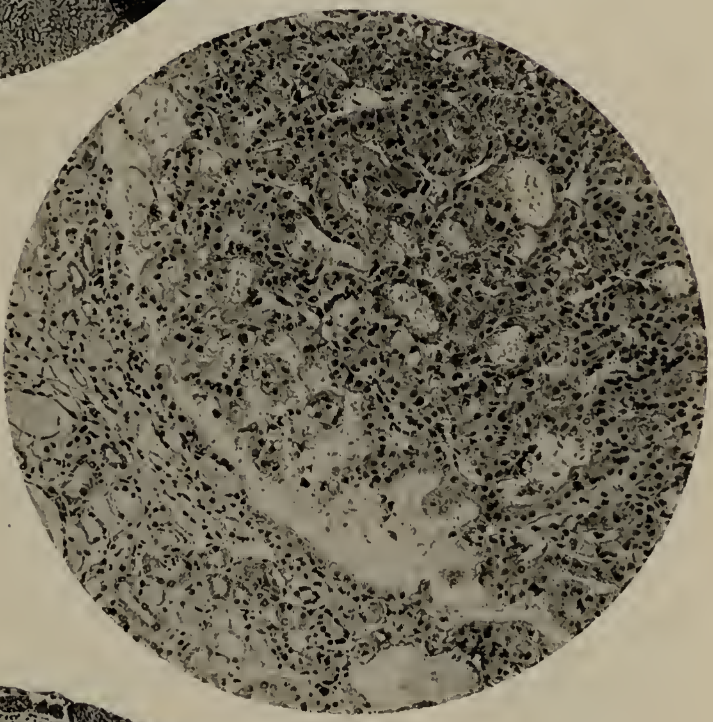

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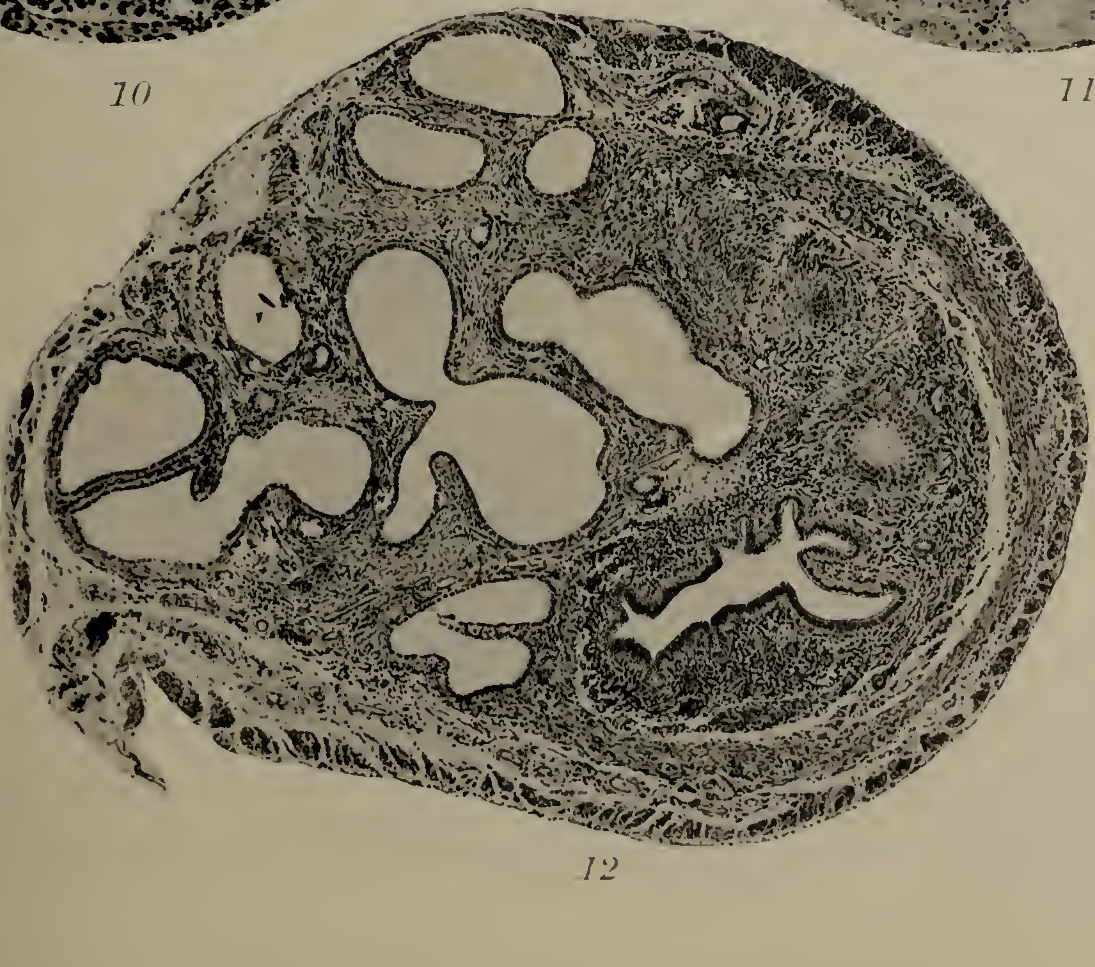

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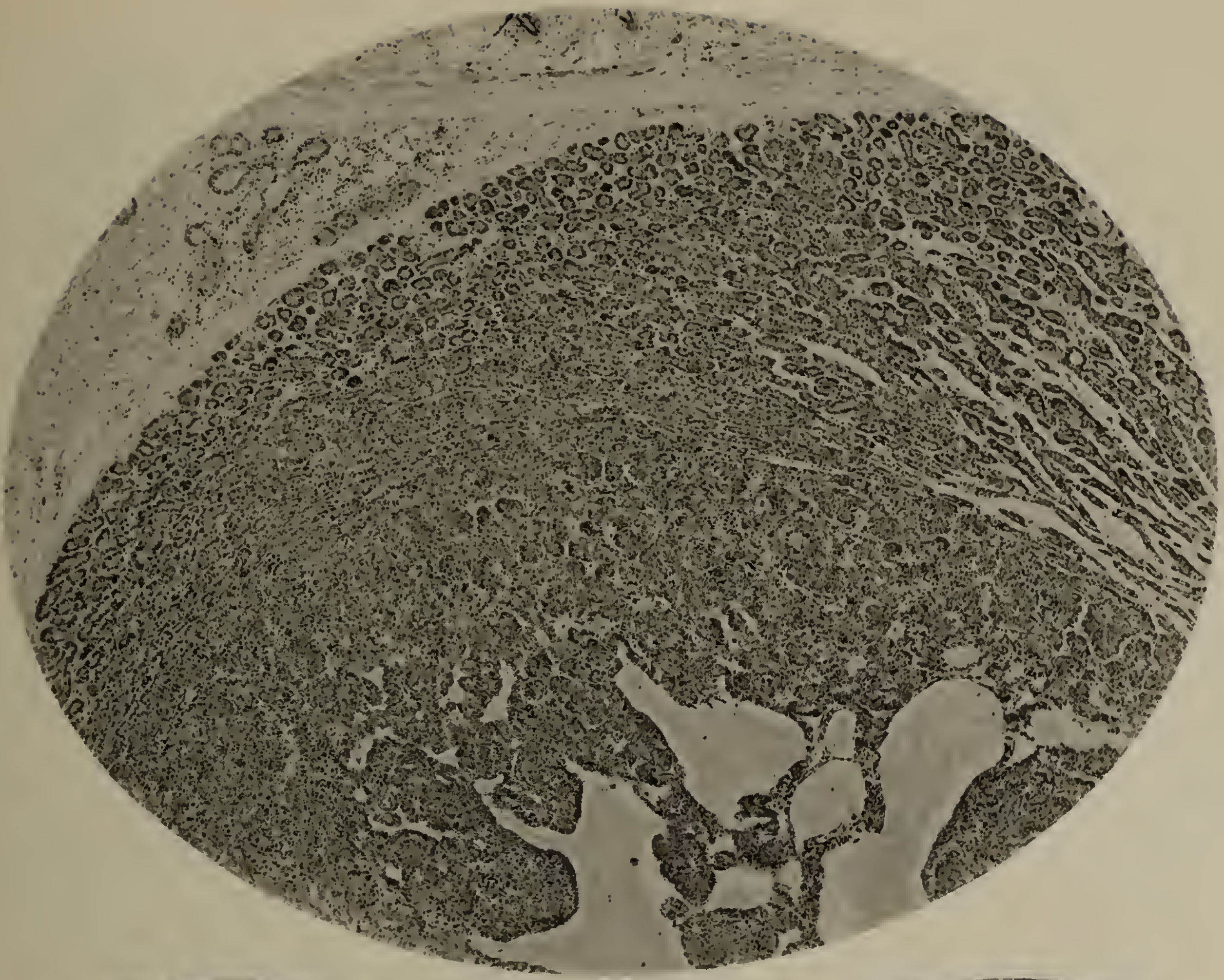

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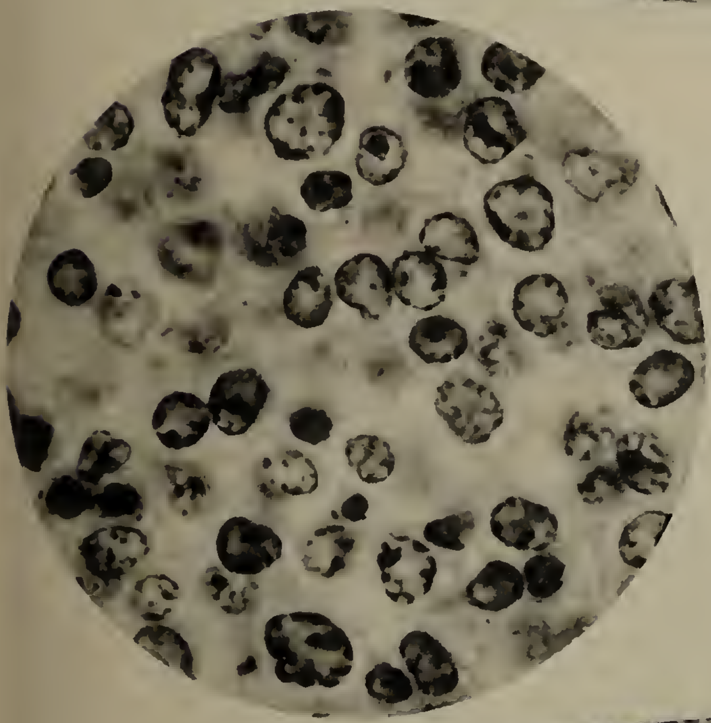

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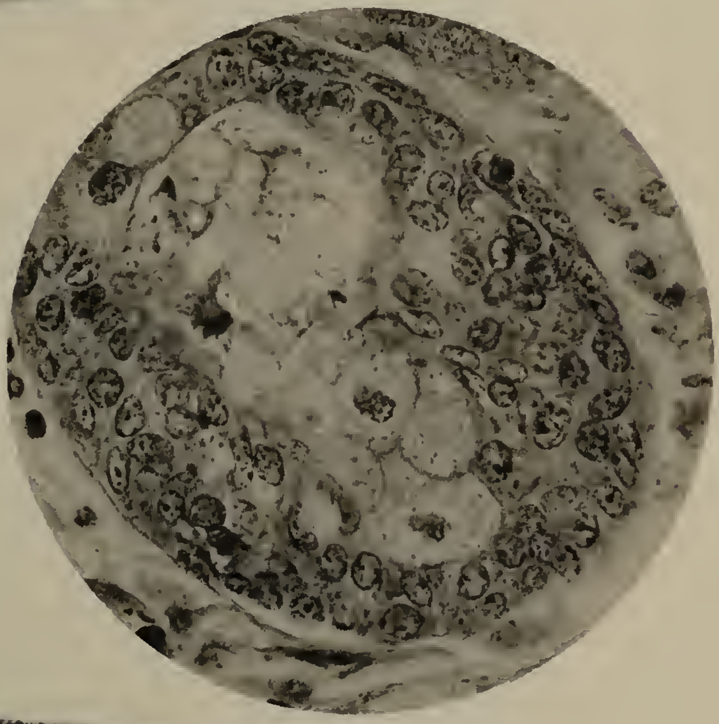

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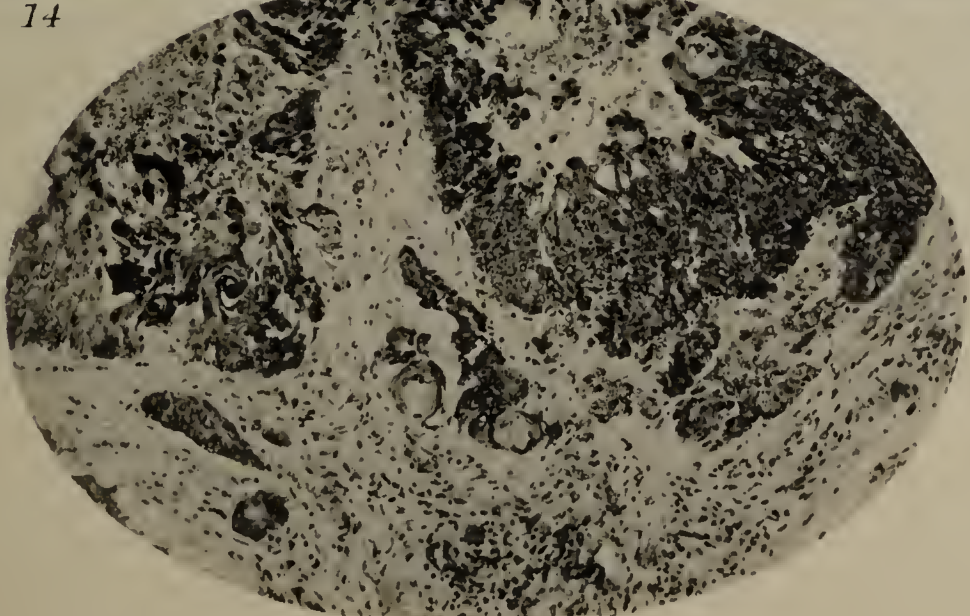

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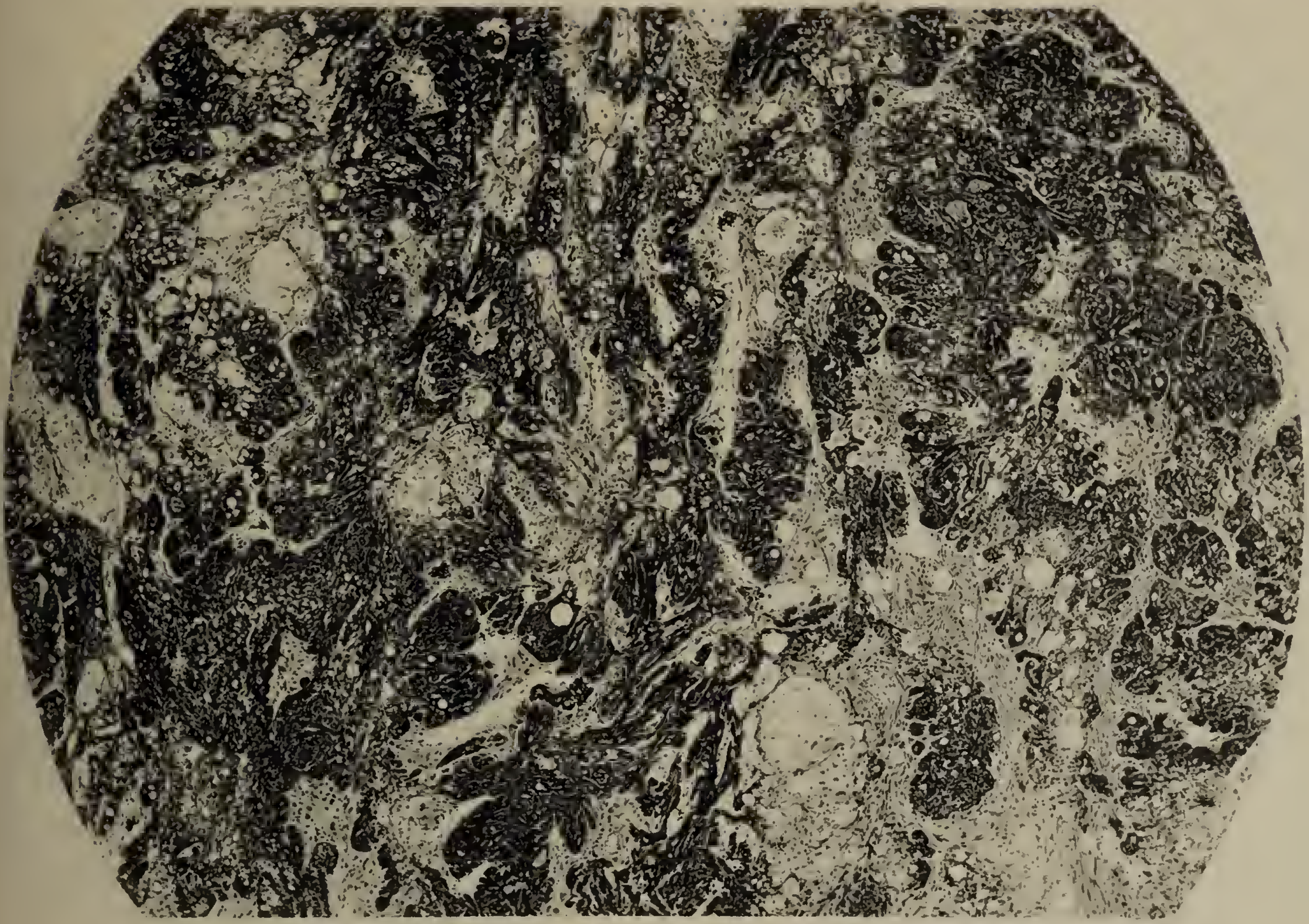

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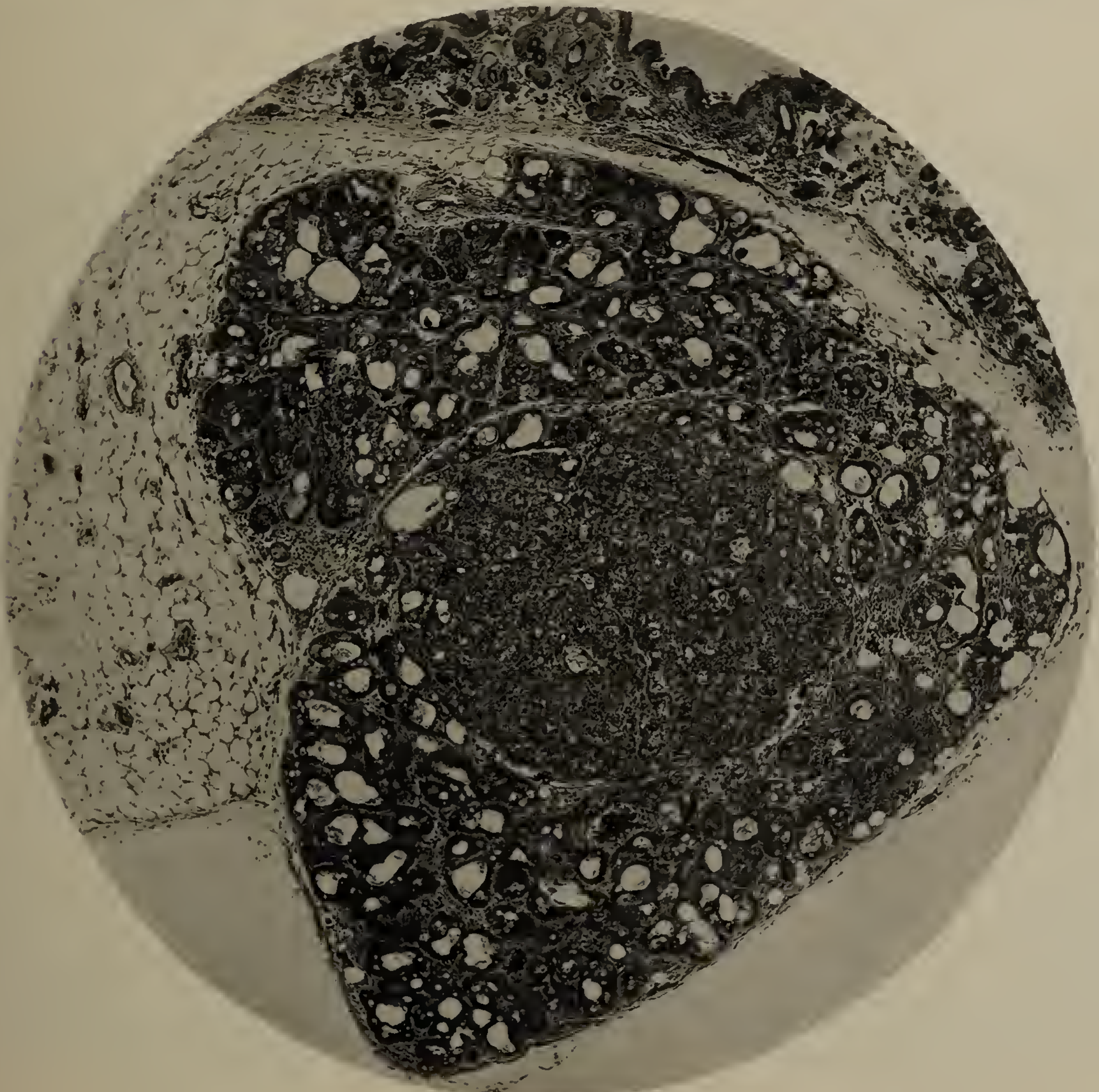

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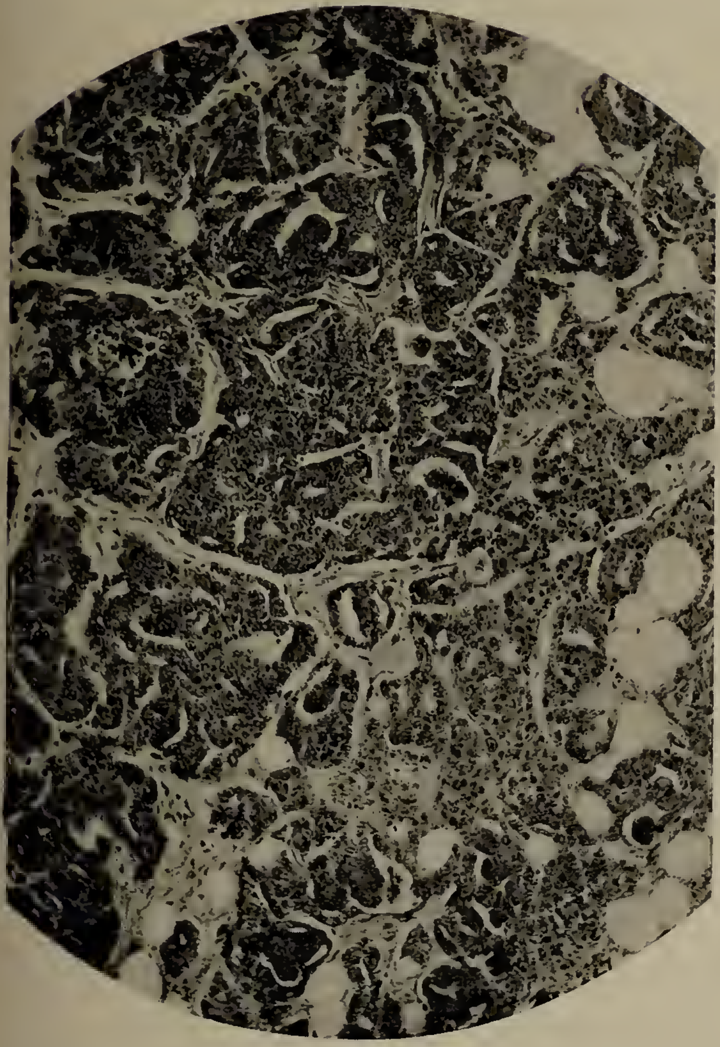

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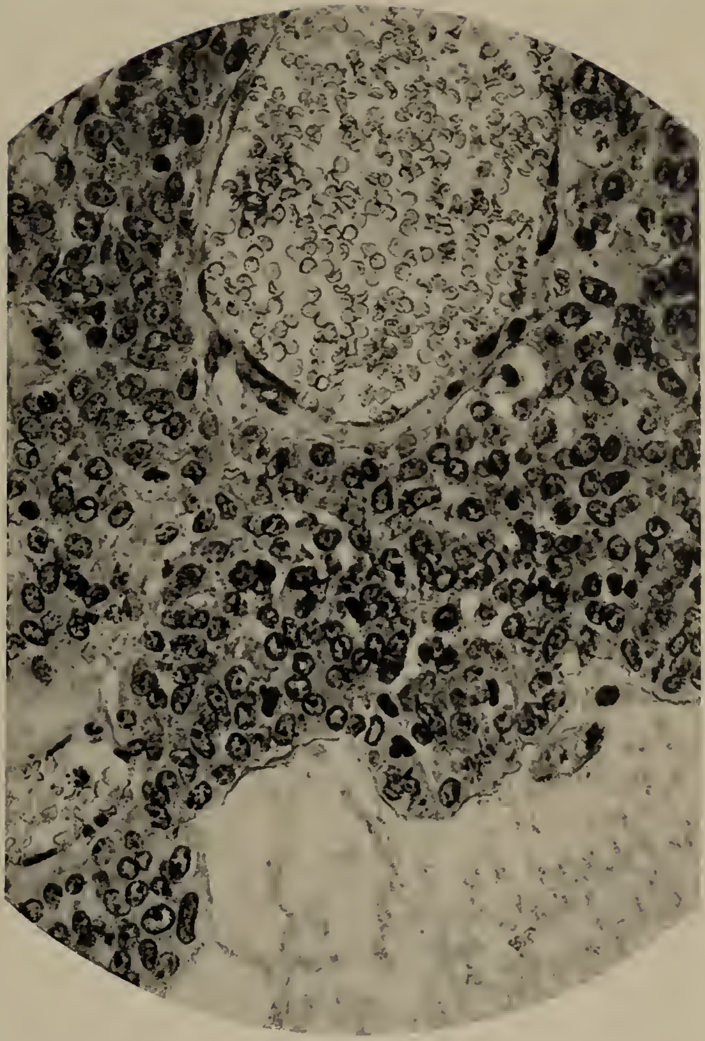

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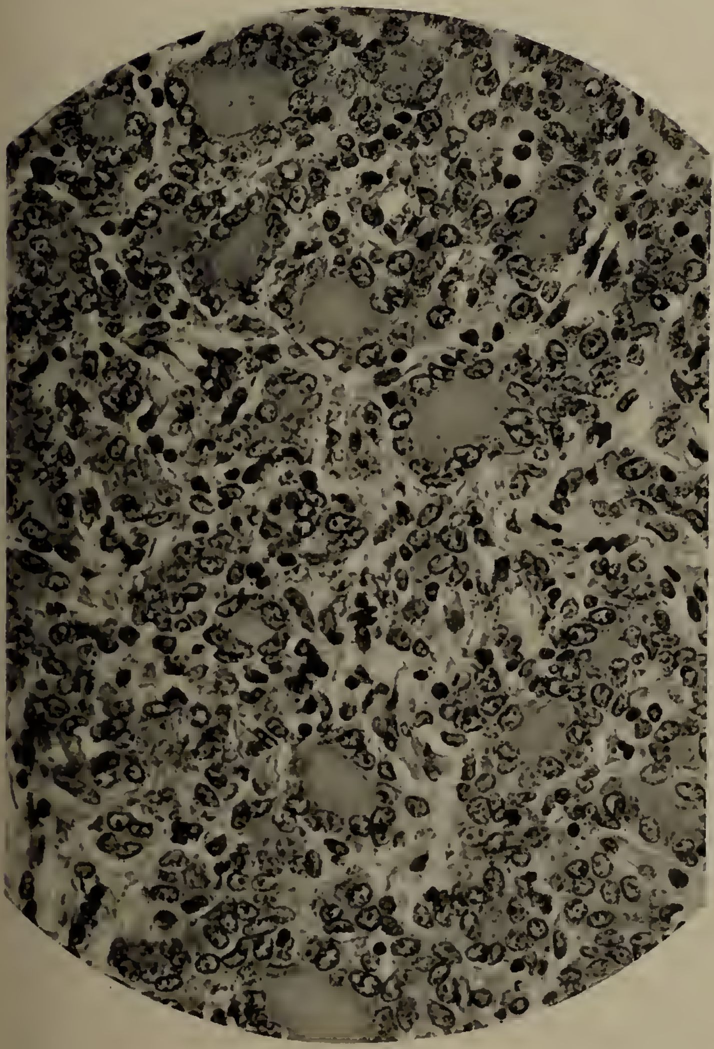

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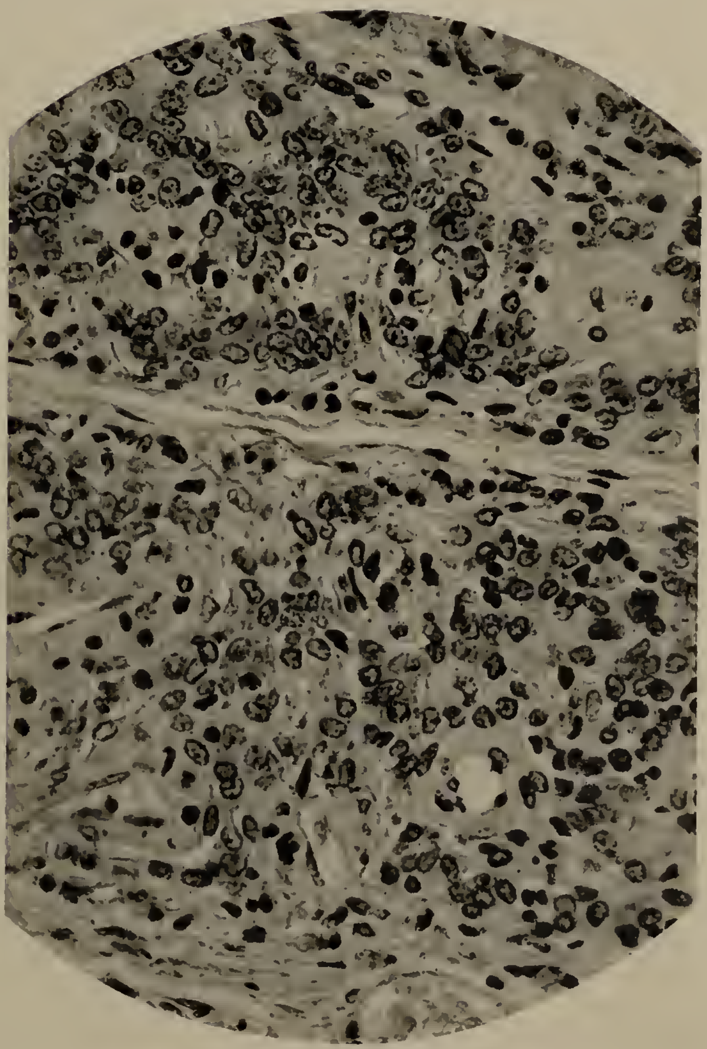

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# A STUDY OF HEREDITY IN RELATION TO THE DEVELOPMENT OF TUMORS IN MICE.*

E. E. Tyzzer.

The statistical methods which heretofore have been applied in the study of the occurrence of cancers in human beings have not as yet furnished proof that the development of these tumors is due to a specific inherited character. In certain families,[1, 2] however, malignant tumors have been so frequent as to attract attention, and the literature bearing upon this subject is considerable. A notable example is found in the family of Napoleon Bonaparte; his father, one of his brothers, and two of his sisters succumbed to cancer of the stomach, as did he himself. On the other hand, the analysis of data derived from a large number of human cases has failed to furnish evidence supporting the view that a predisposition to cancer is inherited.[3] It seems important that the investigation of this question should be carried further, and that use should be made of the experimental method. Since mice breed so rapidly and so frequently develop tumors, they are especially adapted to experimentation of this sort. By regulating the breeding of these animals it is possible to obtain a large amount of accurate data.

The experimental inoculation of different races of the common mouse (Mus musculus) with certain of the inoculable tumors of this species demonstrates beyond question that the tissues of some races are favorable to the growth of these tumors, while the tissues of other races of mice are not. The question arises, — are the races of mice which are susceptible to the inoculable tumors more liable to spontaneous tumors than are the races which are insusceptible? Since heredity is so important a factor in the development of the inoculable tumors, its influence should not be disregarded in the occurrence of spontaneous tumors. If, by selection, a

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* Received for publication Aug. 29, 1907.

race of animals could be obtained nearly all of which developed tumors, even as animals are bred for color or for long hair or short hair, the inheritance of tumors would be established.

There are, however, many difficulties which arise in the way of any such procedure in the breeding of mice. The presence of an internal tumor cannot usually be determined until the death of the animal. Since it is practically impossible to keep all the progeny of all mice, it often happens that the individuals in which tumors are found on post-mortem examination have no available offspring from which to carry on experimental breeding. Mice, when they have developed distinguishable tumors, are usually incapable of further reproduction. In instances in which it is possible to remove an externally situated, primary tumor by operation, the animal is greatly weakened by the procedure, or metastasis has already taken place. On the other hand it would be only by extreme good fortune that offspring could be obtained of parents both of which possessed tumors. This will at once appear evident when it is considered that the adeno-carcinoma of the mammary gland, the most common of the tumors which develop externally in mice, rarely occurs in the males. Furthermore, the mere rearing of a large number of the offspring from mice with tumors can of itself furnish no definite results. Age must be taken into account and all individuals made to live as long as possible.

If the tendency to develop neoplasms be considered an inherited character, the greatest difficulty of all arises in its determination. It is well known that characters exist where they are not distinguishable; thus the color character in certain mammals is present in the embryo, even in the fertilized ovum, although it may be impossible to determine it until later under the conditions of extra-uterine life. Likewise it would not appear improbable that there might be a "tumor character" in certain individuals in which it was not in evidence, but which would possibly become apparent under certain conditions such as those of the menopause or of advanced life.

There is evidence to show that various influences are concerned in the atypical growth of animal tissues. The excitants may be primarily chemical, or mechanical, or of the nature of certain forms of rays and emanations. Concerning the latter, the frequency of carcinoma on the hands of Roentgen-ray operators is well known. Also in certain cases of melanoderma, which closely approaches a neoplasm in its nature, the condition is greatly influenced by light, and the exposed portions of the skin are most affected. While the part played by such external influences is not to be discredited, it is necessary to consider also the possibility that certain individuals possess a tumor character which, under usual or what might be termed "normal" influences, such as are exemplified by light rays in case of melanoderma, may become apparent in the development of a neoplasm. That is, in the origin of the tumor the inherited character constitutes the specific factor.

Whether the tendency to the frequent development of tumors is based on one general or on various specific tumor characters is a question to be considered. In the one case the tumor character will be revealed in the development of tumors of various types; in the other, in the development of tumors of a single type.

The occurrence of cell "rests" or misplaced tissue may possibly have some relation to the inherited tumor character.

If, on the hypothesis that the tendency to the development of tumors is inherited, use is made of the principles of Mendel's law, it becomes at once apparent that the tumor character cannot be a dominant one. If the tumor character should be dominant, then the breeding of an insusceptible individual with one having a tumor would result in hybrids in all of which the tumor character would be dominant. Such a condition as this would be so obvious that it could not fail to have been recognized long before now. If the tumor character is recessive, then the hybrids resulting from the union of an insusceptible individual with one with a tumor character will not in any case show tumors. When, however, these hybrids are bred *inter se*, we should expect twenty-five per cent of the individuals to present the recessive or tumor character. Thus, if (a) stands for the tumor character and (A) for the "no tumor" character, then according to the well-known formula the breeding of two individuals, one with character (A), the other with character (a), results in hybrids Aa, Aa, Aa, Aa, etc., the union of any of which furnishes AA, 2Aa, aa. Thus it is seen that, if the tumor character exists as such, it will most probably be recessive rather than dominant.

It is also possible that the development of a tumor depends upon a combination of several inherited characters rather than upon the presence of a single character. If such is the case it may prove a difficult matter to discover any influence of heredity in the growth of tumors.

That the color character has no relation to the development of tumors is evident from the fact that tumors have been found in white, brown, black, gray, and pied mice.

The following breeding experiments were begun in August, 1905, when a mouse was found with a large tumor of the lung.* The object was primarily to determine whether certain families, as shown by the offspring of mice with tumors, were more liable to develop spontaneous tumors than others. Tumors were subsequently found in other mice, and their offspring were also used for breeding purposes. The method followed has been to keep a record for each mouse, noting the date of birth, description, sex, color, and any apparent disease or change in the general condition which occurs at any time during the entire life. The mice are kept under good hygienic conditions, and are made to live as long as possible. They are killed only when death appears imminent.

Three families of considerable size have been derived from three mice in which tumors developed spontaneously. All three tumors were in races susceptible to the inoculable tumors. As a control a race of mice was taken in which the inoculable tumors had always failed to develop.

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* A similar line of investigation has been suggested by Bashford. The Lancet, 1906, CLXXI., 305.

Since in two families the observations are not as yet ended, the data thus far collected are not sufficient to allow of any conclusions. It is only with the third family that the observations are complete.

Family A. This consists of the offspring resulting from the union of a black female mouse * (Harvard origin) which developed a large papillary cyst-adenoma of the lung, with a white male of a race (Providence origin) insusceptible to the Jensen tumor. The female was black and the male white, so that inheritance as regards the color character could readily be followed in the offspring. Of the young of this pair certain ones were black and others white, so that the female must be considered a hybrid as regards color, for if she had been pure black all the young would have been black, since black is dominant over white. Two young were reared by the female just before the tumor caused her death, and it is from these two that the entire family has been derived.

In certain instances mice of the same generation were bred together, in others mice of different generations.

After the family numbered one hundred and one, breeding was stopped and the mice are now kept under observation. Thirty-six of the total number have died. In none of these were there any tumors found. Four of this number, three males and one female, were over six months old, the oldest being about eleven months at the time of its death, but most of the others were young mice which died before they became fully mature. Six mice were partially eaten by others, so that it was impossible to determine whether there was any internal tumor present.

Of the living mice of this family, one male has attained the age of two years, another male and female one year and six months, and a male one year. All the others are less than a year old, and the majority are not over seven months.

Although up to the present time no tumor has been found in this family, the age thus far attained by any of its members is not sufficiently great to make these results of

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* Case No. 1 of Series of Spontaneous Tumors.

value. The fact that this family is derived from crossing the female presenting a tumor of the lung with a male of a breed insusceptible to the inoculable tumors must be considered. About forty per cent of the offspring are black, thus presenting the color character dominant in the original mongrel female with the lung tumor, but most of these are hybrids, and no more than five are pure black.

A lympho-sarcoma has developed in one of a lot of mice obtained by mating a member of Family A with a mouse of Bashford origin.

Family B. A brown female* (Harvard origin), mated with a white male mouse of another stock (Buffalo origin), reared thirteen offspring. Most of these were used for inoculation and were found susceptible to both the Jensen and the Ehrlich "Stamm 11" tumors. The Jensen tumor in one of these mice† grew very slowly, and, when the mouse was killed, at the age of five and a half months, a primary adeno-cystoma was found in the lung. Of the other twelve mice two escaped, five were ten or more months old when they died, and the remainder died when a few months old. None of these developed primary tumors.

When the male from which this stock originated died, the female was mated with one of the young male offspring. Before the two young offspring resulting from this union were fully mature, the mother developed a lympho-sarcoma (Case No. 11).

The offspring resulting from the union of the female which developed lympho-sarcoma with one of its male young now number eighteen. All but one of these are alive and one year is the maximum age attained.

In this family which has been derived from a female mouse having a lympho-sarcoma, thirty-one offspring have been reared, and twenty-nine have been kept under observation. Six of these died at ages ranging from ten and a half months to fifteen months, but developed no tumors. One of the others, which had been inoculated with the Jensen tumor, died at the age of five and a half months, and presented a primary tumor of the lung. This was six months previous to the development of the lympho-sarcoma in its mother. Of the living members of this family one is a year old, the others considerably younger.

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* Case No. 11 of Series of Spontaneous Tumors.

† Case No. 2 of Series of Spontaneous Tumors.

Family C. The pair of mice from which this family originated were purchased of a dealer in Detroit when quite young and were probably brother and sister of the same litter. Their offspring were found susceptible to the inoculable tumors, and many of them were thus used for inoculation purposes. On the death of the male, at the age of fourteen months, a minute tumor was found in the lung.

The offspring of this pair of mice number twenty-four. All are dead, and a post-mortem examination has been made of each mouse. Tumors of the lung, identical in type with that of the original male, were found in three of the offspring, one in each of the three generations. Thus in the entire family of twenty-six mice there were four tumors of identical character.

Two of the tumors occurred in mice previously inoculated, one with the Ehrlich "Stamm 11" tumor which grew to a large size, the other with a lympho-sarcoma which was never successfully inoculated. The other two tumors which occurred in this family were in mice which had never been inoculated.

The tumors were equally represented in both sexes, two occurring in males and two in females. The entire family consists of thirteen males and thirteen females.

Two of the mice, a male and a female in which the tumors developed, were between eight and nine months of age, the other two, a male and a female, were over a year old. The former two had never been inoculated, the latter two had been inoculated with tumor tissue.

Of the remaining twenty-two negative mice, eleven were never inoculated; two were inoculated with the Jensen tumor which grew in both, eight were inoculated with the Ehrlich "Stamm 11" tumor which grew in three, and one was inoculated with a lympho-sarcoma with a negative result.

The ages attained by the negative mice ranged from one to fourteen months.

The tumors were found on the following dates: May 10, July 10, July 23, October 22, of the same year.

Hybrids of Family C. The results of cross-breeding members of Family C, which represents a pure stock, with other mice are also to be considered. Mice of various other stocks, and in some instances mixed stocks, were used in this cross-breeding. Forty-four of the resulting offspring have been autopsied. All but one of these had been previously inoculated with either the Jensen or the Ehrlich "Stamm 11" tumor. Most of the mice in which the inoculated tumors

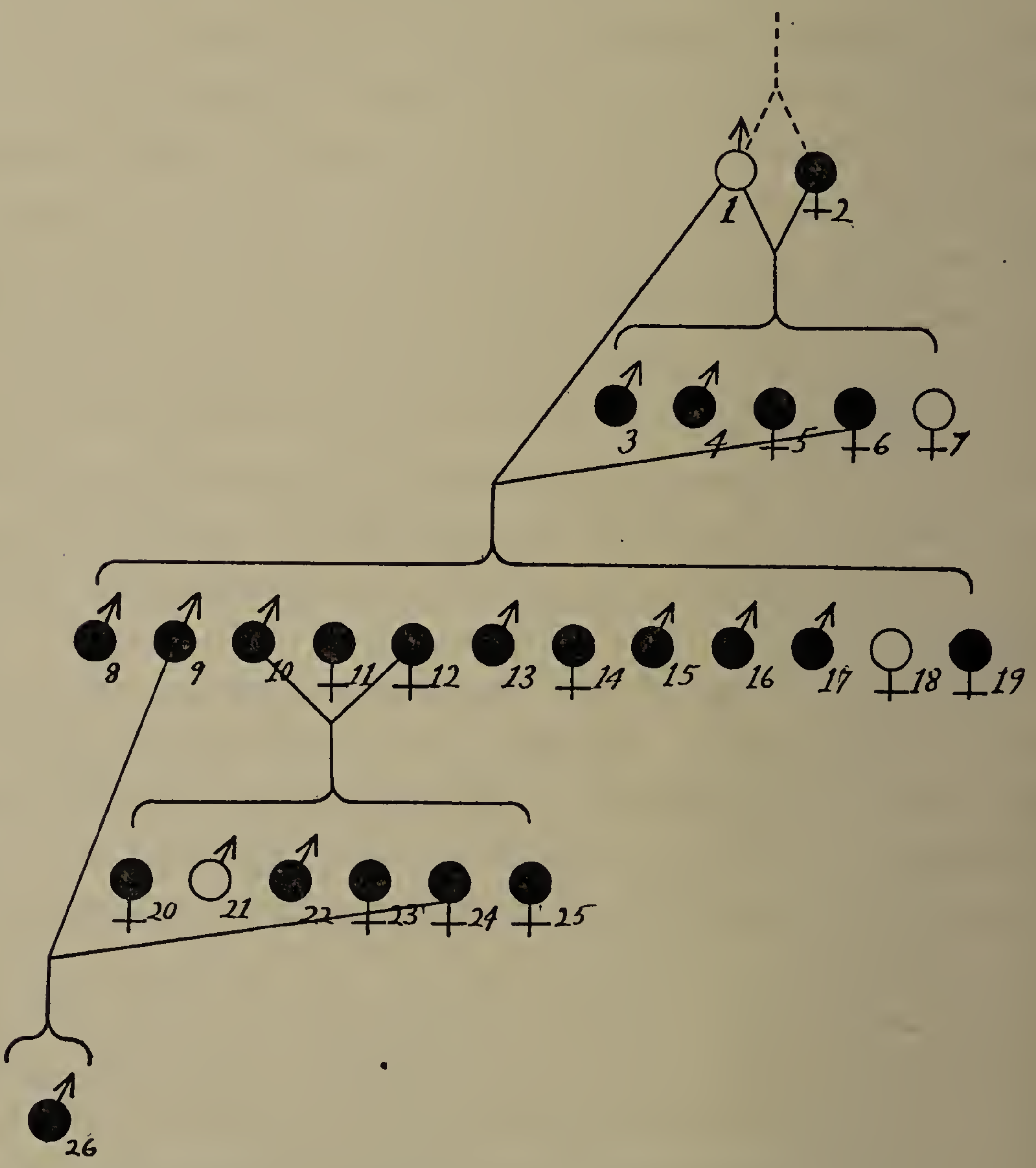

Chart showing the occurrence of tumors in Family C:
○ = mice which developed tumors.
● = mice in which no tumors were found.

grew succumbed at an early age, only two living to six months. Many of the mice in which the inoculated tumors failed to grow lived to a much greater age.

One, a hybrid female, the offspring of a member of Family C (Detroit origin) and a mouse derived from the union of a member of Family A with a mouse of the stock of the ancestral female of that family (Harvard origin), had never been inoculated. It died at the age of eight months and presented a primary papillary cyst-adenoma of the lung* similar to those found in the two families from which it was derived.

Of the forty-three inoculated hybrids which had been derived by mating certain members of Family C with mice of various stocks, two † presented similar tumors of the lung. These were two females of a single litter resulting from mating a male of Family C with a hybrid female, derived from the union of mice of two different stocks (Harvard and Bashford), in both of which tumors of the lung have occurred. Both of these mice had been inoculated with the Jensen tumor. In one a nodule appeared at the site of inoculation, grew for a time, but was later on absorbed. Both of these mice in which the tumor of the lung was found died when slightly less than a year old.

Thus, primary tumors of the lung occurred in only two of the forty-three inoculated hybrids, whereas the only uninoculated hybrid presented a tumor of this type. From these data and from the infrequency of primary tumors in a long series of mice inoculated with various types of tumors, there does not appear to be any relation between such inoculations and the development of primary tumors. In order, however. to obtain more data on this point, it might be well to repeat the procedure followed in Family C, and inoculate one-half the members of other susceptible families so as to determine the frequency of primary tumors in equal numbers of inoculated and uninoculated mice of the same family.

Family D is derived from mice purchased from a

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* Case No. 7 of Series of Spontaneous Tumors.

† Cases Nos. 8 and 9 of Series of Spontaneous Tumors.

Washington dealer. Since the inoculation of these mice with either the Jensen or the Ehrlich "Stamm 11" tumors gave invariably negative results, the family was taken as a control for the hypothetically susceptible families A, B, and C. This family has been kept under observation for over two years and a careful post-mortem examination has been made of all mice that have died. No tumor has been found in any case. Of twenty-three that have died, two were more than a year old and all the others were adults of unknown age. The members of this family are kept together in two large cages, which are seldom cleaned and then not very thoroughly, so that the ideal conditions are furnished for cage infection.

In working up the data of these breeding experiments a mistake has been found in that the experiments were made too complicated. Thus where individuals of different generations have been bred together, the experiment becomes too involved for the interpretation of results.

The fact that the character in question is, in many cases, not apparent adds to the difficulty. These experiments are thus to be considered as being somewhat preliminary in nature, but indicate, nevertheless, a line of work which is certain to lead to definite results whether positive or negative; that is, it seems possible by experimental procedure to determine whether there is a tumor character inherited in accordance with Mendel's law.

It has already been pointed out that if such a character exists it is probably recessive. In this case if two animals with the tumor character are mated together all the offspring should possess the tumor character. This is possibly the case in Family C, but it is then necessary to assume that the tumor character, present in all the mice of this family, became apparent only in four individuals.

According to Mendelian principles, if one of the parents possess the recessive tumor character and the other the dominant "no tumor" character, the first generation of offspring would be hybrids in all of which there would be no evidence of the recessive tumor character. However, by breeding these hybrids together, twenty-five per cent of the young would possess only the "no tumor" character, fifty per cent would be hybrids with the "no tumor" character dominant, and twenty-five per cent would possess only the recessive tumor character. On this basis it will not be difficult to compare, as regards the development of tumors, the second generation of the offspring of an insusceptible male and a tumor female with the offspring obtained by breeding the same male with an insusceptible female. A breeding experiment of this sort has just been commenced.

The results of the previous breeding experiments may be briefly summarized as follows:

Family A, which has been derived from a female mouse, with a papillary cyst-adenoma of the lung, mated with a male of a stock insusceptible to the inoculable tumors, has furnished no offspring presenting tumors. The observations are, however, not yet complete since the majority of the mice are still alive and many are still young.

Family B originated by mating a female which developed a lympho-sarcoma with a male of a stock susceptible to the inoculable tumors. This also furnishes rather meager data, since the mice now living are relatively young, and most of those already dead succumbed at an early age. One of the offspring, however, which had been inoculated successfully with the Jensen tumor, presented also a papillary cyst-adenoma of the lung at the age of four and a half months. In this instance the primary tumor in the offspring was of a type distinct from that of the tumor of the parent.

Family C originated with a pair of mice, evidently of the same litter, the male of which presented a minute papillary cyst-adenoma of the lung. In this family three of the twenty-four offspring developed tumors of the lung of the same type as that which occurred in the first male.

The cross-breeding of certain members of Family C with mice of other stocks has resulted in offspring, forty-four of which have been autopsied. All but one of this number had been previously inoculated with either the Jensen or the Ehrlich "Stamm 11" tumors. The one uninoculated mouse and two of the forty-three inoculated mice have developed tumors of the lung similar to those found in Family C.

Family D, which consists of a race of mice insusceptible to the inoculated tumors, has not yet furnished an example of a spontaneous tumor.

All these tumors occurred in mice which were not extremely old, so that their frequency is the more important.

That the frequency of occurrence of the papillary cyst-adenoma of the lung is the same in the noninoculated as in the inoculated mice is indicated in Family C, in which an equal number of each at about the same age may be compared.

It seems possible that heredity may explain the occurrence of tumors among mice kept in certain cages, instead of cage infection, as has been the belief of several investigators. If infection or other conditions have some remote causal relation to the development of tumors, then the relatively great susceptibility of certain families and the insusceptibility of others to spontaneous tumors is indicated thus far in the breeding experiments. This is especially applicable to the occurrence of the papillary cyst-adenoma of the lung. Although the conditions furnished the family of control mice were most favorable for infection, yet no tumor has developed among them.

Families which show greatest insusceptibility to the inoculated tumors are less liable to spontaneous tumors, although this may possibly not always hold true for individuals. As regards the latter question, however, it is difficult to determine, in a mouse in which a primary tumor is found after an inoculated tumor has failed to grow, whether the primary tumor was already in existence before the inoculation or developed subsequently. Although in certain cases the lung tumors were found many months after the failure of the inoculable tumor to grow, they may possibly have been so slow of growth as to have existed at the time of the inoculation. On the other hand, the failure of the inoculable tumor to grow does not invariably indicate the insusceptibility of the animal, for in a certain number of cases second inoculations have been successful.

In conclusion it may be stated that the results of these breeding experiments are not thus far sufficiently numerous to prove or disprove that the development of a tumor is dependent upon the presence of an inherited character, although the data obtained may appear to favor this view. It seems possible by certain lines of experimental breeding, with the principles of Mendel's law of heredity in mind, to obtain definite results, whether they be positive or negative.

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THE

# CAROLINE BREWER CROFT FUND CANCER COMMISSION

## HARVARD MEDICAL SCHOOL

Volume I.
Reports Nos. 1, 2, 3 and 4

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BOSTON
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1907

# CAROLINE BREWER CROFT FUND CANCER COMMISSION

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